STUDIES IN THE PHYSIOLOGY OF THE FUNGI.

XVII. THE GROWTH OF CERTAIN WOOD-DESTROYING FUNGI IN RELATION TO THE H-ION CONCENTRATION OF THE MEDIA[1]

F. S. WOLPERT

Formerly Rufus J. Lackland Fellow in the Henry Shaw School of Botany of Washington University
Chairman of the Botany Department, The Principia, St. Louis

INTRODUCTION

That wood-destroying fungi grow more favorably upon substances acid in reaction has been appreciated by many students who have given attention to the biology of these organisms. Investigations in this field have received a marked impetus from the recently perfected methods for determining the hydrogen-ion concentration of solutions. Webb ('19, '21) has given many interesting data upon the effect of hydrogen-ion concentration upon spore germination, while other investigators from this laboratory and those from other laboratories have worked upon the relationships of hydrogen-ion concentration of the medium to the mycelial growth of some of the *Agaricales*.

However, since this order of the fungi contains such a large number of wood-destroying species, and because increased knowledge of their biology leads to better methods of combating their spread and ravages, through improved methods of preservation from a practical as well as from an academic standpoint, it seems advisable to make a more detailed study of the following questions: What hydrogen-ion concentration will inhibit mycelial growth of these fungi? How do these fungi react to hydrogen-ion concentration in different types of media and at different temperatures? Is growth always inhibited by an alkaline solu-

tion? What are the changes in the hydrogen-ion concentration of the substratum caused by mycelial growth? The present paper attempts to answer these questions as completely as the available time would allow.

HISTORICAL REVIEW

Tubeuf ('03) found that NaH_2PO_4 in solution supported mycelial growth of *Merulius lacrymans*, while Na_2HPO_4 and Na_3PO_4 did not. Similar results were obtained with the acid and alkaline phosphate salts of potassium and ammonium. As the monobasic salts are acid and the dibasic and tribasic salts are alkaline, it is concluded that growth is inhibited in alkaline solutions but not in acid solutions.

Rumbold ('08) was the first to determine the inability of the *Agaricales* as a group to grow upon alkaline media. The acidity of separate portions of 5.0 per cent gelatine and 0.5 per cent malt extract solution was so adjusted that solution 1 reacted red to litmus paper; solution 2, red-violet; solution 3, violet; solution 4, blue-violet; and solution 5, blue. Fifteen species of *Agaricales* including *Schizophyllum commune*,[1] *Lenzites sepiaria*, *Pholiota adiposa*, and *Armillaria mellea* grew extensively in solutions 1 and 2, somewhat less in solution 3, still less in solution 4, and not at all in solution 5. Solutions 1, 2, and 3 were considered by her to be acid; solution 4, neutral or very slightly alkaline; and solution 5, distinctly alkaline. It is known that litmus gives a positive red color in solutions which have an active acidity of P_H 5.0 or greater, a red-violet color when the P_H is approximately 6.0, and a violet color close to P_H 7.0. A blue color is obtained in all solutions with an active alkalinity of P_H 8.0 or greater. From this it is concluded that, while the organisms used by Miss Rumbold did grow to some extent in a neutral or slightly alkaline solution the amount of growth was less than that obtained in solutions with initial acidities of approximately P_H 5.0 and 6.0. Furthermore, growth was entirely inhibited in solutions which were as alkaline as P_H 8.0.

These same fungi grew rapidly in an acid medium consisting of: 100 cc. Knop's solution, 3 gms. agar, and 5 gms. grape sugar.

[1] The recent and prevalent names have been used for all fungi under discussion.

Good growth resulted when 0.25 per cent of sulphuric acid was added to this solution. When the same medium was made alkaline with sodium carbonate, no growth was obtained.

The acidity of the medium has been shown by Falck ('12) to be a conditioning factor for spore germination and for mycelial growth of species of *Merulius*. Furthermore, he has observed that growth and development of *Merulius* is more rapid upon wood previously infected by *Coniophora cerebella* than upon sound wood. Since mycelial growth of *Coniophora cerebella* makes the substratum decidedly acid, it is assumed that species of *Merulius* are partial to acid media.

Wehmer ('14) indicated that *Merulius lacrymans* increased the active acidity of the medium upon which it grew. One gm. of sound pine wood pulverized and boiled in 30 cc. of water and titrated against 0.1 N NaOH[1] required 0.9–1.2 cc. to neutralize the solution. Decayed wood similarly treated required 4.9–8.0 cc. for neutralization.

Lenzites sepiaria, *Fomes pinicola*, *Polystictus versicolor*, and *Polyporus lucidus*, according to Zeller ('16), did not grow upon slightly alkaline Thaxter's glucose-potato-hard agar, while a readjustment to slight acidity resulted in good growth. This indicates for all of these species a marked intolerance to alkaline media.

The hydrogen-ion concentration necessary to inhibit the growth of *Lenzites sepiaria*, *Fomes roseus*, *Coniophora cerebella*, and *Merulius lacrymans* upon synthetic and malt extract media was determined by Meacham ('18). While the 4 species show considerable fluctuation, they respond in much the same way to active acidity, showing maximum growth at approximately P_H 3.0. A composite curve indicates good growth with increasing acidity until P_H 3.0–2.6 is reached. There is a distinct critical zone between P_H 2.6 and 1.9 where growth decreases rapidly, followed by total inhibition at P_H 1.7. In general, these figures indicate a marked tolerance to high acidity.

Schmitz ('19) studied the hydrogen-ion concentration conducive to optimum growth for *Fomes pinicola*, *Lenzites sepiaria*,

---

[1] It is assumed that this solution was NaOH, since Wehmer indicated it as a 1/10 N. N. solution.

and *Polystictus versicolor* upon a carrot extract-glucose-agar medium. His results, expressed in the diametric growth in centimeters of the fungus colonies, show that for the first 2 species there is little difference in growth from $+ 5$ to $+ 24.5$, Fuller's scale, while at $+ 2.5$ there is a marked retardation. *Polystictus versicolor*, on the other hand, is more sensitive to changes in acidity, showing maximum growth at $+ 9.75$ and a steady decrease as the acidity increases. He concluded that slight variations in the acidity of the substratum did not affect the growth of *Fomes pinicola* and *Lenzites sepiaria*, while they might influence that of *Polystictus versicolor*.

Employing Czapek's, Dunham's, Reed's, and Richards' solutions, sap from *Acer saccharinum*, and a pine decoction, a determination of the influence of the hydrogen-ion concentration, among other things, upon the growth of wood-destroying fungi was attempted by Zeller, Schmitz, and Duggar ('19). Although it is impossible to make any conclusive statements, within the range of the experiments the hydrogen-ion concentration was not a limiting factor in growth. The control solutions showed that in only one series, the Czapek's solution with K_3PO_4, was the reaction definitely alkaline with an initial P_H of 8.6 at the time of inoculation. Upon this solution *Polystictus versicolor* grew slowly, changing the reaction to P_H 4.8 within 30 days. *Daedalea confragosa* failed to grow upon this same solution, the final reaction being P_H 8.4. These results suggest that all of the wood-destroying fungi do not react alike toward slightly alkaline solutions.

Webb ('19) studied spore germination of a number of fungi in relation to the hydrogen-ion concentration of a $M/5$ mannite medium. Spores of *Lenzites sepiaria* did not germinate readily when the reaction was acid. Increasing acidity from P_H 7.0 to 3.1–2.8 favorably affected germination of the spores of this and of other species of fungi.

A second paper by Webb ('21) showed that increasing acidity of mannite, peptone, and Czapek's solutions, sugar beet decoction, "water H_3PO_4 and NaOH," and "water HCl or KOH" from neutrality to approximately P_H 3.0 to 4.0 favorably influenced spore germination of *Lenzites sepiaria* and of other

fungi. Beet decoction gave the best percentage and range of germination and "water H_3PO_4 and NaOH" the poorest. He observed that in equal concentrations the OH ions are more toxic to the spores of the fungi studied than are the H ions. His results indicate that the range and percentage of germination, as influenced by the hydrogen-ion concentration, depend upon both the organism and the medium, and that the direction and magnitude of the change in the reaction of the medium due to spore germination depend upon the fungi, the medium, and the initial reaction of the solution.

METHODS

Organisms.—In the selection of organisms, 3 things were considered: (1) the use of as many representative genera as possible, (2) the use of species found commonly both on deciduous woods and on coniferous woods, and (3) the use of forms which grow well upon artificial media. With these considerations in mind, the following 8 species were selected: *Polyporus adustus* (Willd.) Fr., *Polystictus versicolor* (L.) Fr., *Pleurotus ostreatus* Jacq., *Schizophyllum commune* Fr., *Pholiota adiposa* Fr., *Lenzites sepiaria* (Wulf.) Fr., *Armillaria mellea* (Vahl) Quel., and *Daedalea confragosa* (Bolt.) Fr.

These species are common wherever their respective hosts are found. Preliminary work has shown them to make more rapid growth in artificial culture than many other common fungi. Weir ('14) found that *Armillaria mellea* is common both on deciduous and on coniferous woods; that *Lenzites sepiaria* is confined almost entirely to coniferous species, whereas *Polystictus versicolor* and *Polyporus adustus* are usually upon deciduous species but are found occasionally upon coniferous hosts. *Pholiota adiposa* is found on *Abies grandis* as well as on some deciduous trees. *Schizophyllum commune*, common on deciduous woods, according to Rhoades ('21), occurs occasionally upon coniferous hosts. *Daedalea confragosa* and *Pleurotus ostreatus* are regarded as saprophytic upon deciduous woods.

Pure culture methods.—Pure cultures were made by employing either the tissue-culture method described by Duggar ('05) or the spore method used by Zeller ('16). These methods have

been adequately described in the papers referred to and in later papers from this laboratory, so they require no discussion at this point. The spore method was used when it was impossible to secure sterile tissue, as from particularly thin sporophores. After the mycelium had made considerable growth upon potato agar, the cultures were transferred to large bottles of sterile bean stems and pods, to sterile decayed wood, and to sterile decayed wood mixed with decayed leaves. Growth was more rapid in the bean stem and pod cultures, but in all cases it was abundant enough to constitute satisfactory stock cultures.

Inoculum was prepared according to the method of Zeller, Schmitz, and Duggar ('19) by growing bits of mycelium from these stock cultures upon plates of sterile agar made according to the following formula: 1000 cc. potato water from 240 gms. of potatoes boiled for 1 hour, 20 gms. cane sugar, 10 gms. KNO_3, 5 gms. KH_2PO_4, and 20 gms. agar. After a growth period of 10 days to 2 weeks, these plates were cut into pieces 8–10 mm. square. Each culture flask received 1 of these squares of inoculum.

Culture solutions.—The culture media can be divided into two classes, based on the absence or presence of celluloses. The first class consists of 3 types, as described later, namely: (1) a modification of Richards' E solution, (2) a peptone-nutrient solution with sugar, and (3) a peptone-nutrient solution without sugar.

The modified Richards' E solution contained: $MgSO_4$, 0.5 gm.; KNO_3, 5 gms.; NH_4NO_3, 10 gms.; trace $FeSO_4$; varying amounts of H_3PO_4, KH_2PO_4, and K_2HPO_4 to give a total of 10.4 gms. of phosphate; and doubly distilled water, 1000 cc. The 3 forms of the phosphate were used in varying proportions to obtain a range of reaction from P_H 2.5 to 8.0 at intervals of 0.5. The large percentage of phosphates and the reduced amount of sugar produced a heavily buffered solution which held, as nearly as possible, the initial P_H throughout the entire incubation period. The amount of $MgSO_4$ was reduced because in the presence of phosphates it produces a precipitate in an alkaline solution. Although all precautions were taken in making the media, slight differences in P_H were evident in each series, requiring some slight variation in the proportion of the phosphate buffers

employed. Table I indicates the method employed in making the solutions.

The nutrient solution, containing all of the nutrients except the phosphates, comprised 28 cc. of the 35 cc. of each culture, or 80 per cent. Consequently all salts and sugar were weighed out on the basis of 1000 cc. of culture solution but made up to only 800 cc. with doubly distilled water. Twenty-eight cc. of this solution were added to each flask. The addition of 7 cc. of phosphate solutions to each culture resulted in bringing all nutrients to the desired concentrations. The KH_2PO_4 was added to the flasks before sterilization; the K_2HPO_4 and H_3PO_4 were autoclaved separately and added aseptically. Sterilization consisted in autoclaving for 15 minutes at 12–15 pounds pressure. This procedure eliminated, as far as possible, any alterations in the acidity of the solutions during autoclaving. The series P_H 8.0 was obtained by adding, before sterilization, 0.5 gm. of $CaCO_3$ to flasks containing 7 cc. of K_2HPO_4. The reaction of this solution varied from P_H 7.8 to 8.2. All hydrogen-ion determinations were made according to the colorimetric method of Clark and Lubs ('17) and Clark ('20).

TABLE I

THE COMPOSITION OF THE MODIFIED RICHARDS' E SOLUTION ADJUSTED TO A RANGE OF P_H FROM 3.0 TO 8.0 AT INTERVALS OF 0.5

Initial P_H	No. cc. M/3 H_3PO_4	No. cc. M/3 KH_2PO_4	No. cc. M/3 K_2HPO_4	No. cc. nutrient solution	Total no. cc.
3.0	1.2	5.8		28	35
3.5	0.5	6.5		28	35
3.9	0.1	6.9		28	35
4.4		7.0		28	35
5.0		6.8	0.2	28	35
5.5		6.3	0.7	28	35
6.0		5.0	2.0	28	35
6.5		3.5	3.5	28	35
7.0		1.5	5.5	28	35
7.6			7.0	28	35
7.8–8.2	0.5 gm. $CaCO_3$ added		7.0	28	35

The peptone-nutrient solution with sugar contained: bacto-peptone, 25 gms.; cane sugar, 30 gms.; $MgSO_4$, 0.5 gm.; trace of $FeSO_4$, varying amounts of H_3PO_4, KH_2PO_4, and K_2HPO_4 to

give a total of 9.65 gms. of phosphates; and doubly distilled water to make 1000 cc. of solution. This peptone-nutrient solution throughout this work will be referred to as the peptone solution. This solution without sugar gave the third type of media used in this first class of solutions. As with the Richards' solution, slight variations in the hydrogen-ion concentrations were encountered, but these were all eliminated by slight modifications of the phosphate content, indicated in table II.

TABLE II

THE COMPOSITION OF THE PEPTONE-NUTRIENT SOLUTION ADJUSTED TO
A RANGE OF P_H FROM 2.0 TO 8.5 AT INTERVALS OF 0.5

Initial P_H	No. cc. $M/3$ H_3PO_4	No. cc. $M/3$ KH_2PO_4	No. cc. $M/3$ K_2HPO_4	No. cc. nutrient solution	Total no. cc.
2.0	7.0	1 cc. conc. H_3PO_4		28	35
2.5	7.0	0.1 cc. conc. H_3PO_4		28	35
3.0	7.0			28	35
3.5	5.0	2.0		28	35
3.9	3.0	4.0		28	35
4.5	2.0	5.0		28	35
5.0	1.0	6.0		28	35
5.6		7.0		28	35
6.0		6.0	1.0	28	35
6.5		4.5	2.5	28	35
7.0		2.0	5.0	28	35
7.4			7.0	28	35
7.8–8.2	0.5 gm. $CaCO_3$		7.0	28	35
8.5–8.7	1.5 gm. $CaCO_3$ 0.2 cc. conc. KOH		7.0	28	35

The nutrient solution was made up and added to the culture flasks in the same manner as in the Richards' solution. Here, too, the H_3PO_4 and K_2HPO_4 were added aseptically.

The second group of nutrient solutions, those containing celluloses as the only or chief source of carbon are: (1) a modified Richards' E solution with a wood cellulose suspension substituted for the cane sugar; (2) a 0.5 per cent peptone-nutrient solution to which is added 20 gms. of filter-paper in strips for each liter of medium; and (3) the Richards' solution modified as in (1) with the addition of 20 gms. of agar.

The celluloses were prepared from longleaf pine, white oak, sugar maple, and poplar woods. Two-hundred-gm. amounts of each wood cut into small chips were treated for one month at

$2°$ C. in a mixture of 520 cc. of KNO_3 of 1.1 specific gravity and 30 gms. of $KClO_3$ (Zeller, Schmitz and Duggar, '19). After washing and precipitating in Schweitzer's reagent, according to McBeth ('16), an abundance of flocculent cellulose was obtained. This was washed repeatedly in distilled water until free from copper. The washing process was hastened by centrifuging and decanting, the necessarily long periods of time required in the usual settling method being avoided.

TABLE III

THE COMPOSITION OF THE MODIFIED RICHARDS' E SOLUTION CONTAINING CELLULOSE ADJUSTED TO A RANGE OF P_H FROM 3.0 TO 6.0 AT INTERVALS OF 1.0

Initial P_H	No. cc. $M/3$ H_3PO_4	No. cc. $M/3$ KH_2PO_4	No. cc. $M/3$ K_2HPO_4	No. cc. nutrient solution	No. cc. cellulose solution	No. cc. total solution
3.0	1.2	5.8		14	14	35
4.0	0.1	6.9		14	14	35
5.0		6.8	0.2	14	14	35
6.0		5.0	2.0	14	14	35

The Richards' solution with cellulose was adjusted to the desired P_H according to table III. As in the previous cases this table required some slight adjustment for each series. The phosphates constitute 20 per cent of the total volume; the cellulose solutions, 40 per cent; and the nutrient salts, 40 per cent. Therefore all salts were weighed on the basis of 100 per cent, or 1000 cc. of solution, and dissolved in enough doubly distilled water to give a total of 400 cc.—40 per cent of the total amount. Fourteen-cc. amounts of this solution were added to each flask. The proper balance was obtained by the addition of 7 cc. of phosphate solutions and 14 cc. of the cellulose suspension to each flask containing this concentrated nutrient salt solution. The KH_2PO_4 was the only phosphate added before sterilization.

The peptone-filter-paper solution was adjusted to the desired initial P_H according to table II. The reduction in the amount of peptone did not change the amount of phosphates required to obtain the initial P_H. The strips of filter-paper were added in equal amounts to each flask. On the basis of 20 gms. of paper per liter of solution, each culture flask received approximately 0.7 gm. of paper.

In the agar series each 1000 cc. of medium contained 500 cc. of the cellulose suspension, 500 cc. of the nutrient salt solution, and 20 gms. of agar. The P_H was adjusted by the addition of M/3 solutions of H_3PO_4 and KOH. To 150-cc. amounts of the warm agar mixture were added:

Series I, 5.0 cc. H_3PO_4, giving approximately P_H 2.8.

Series II, no addition, giving approximately P_H 4.0.

Series III, 2.5 cc. KOH, giving approximately P_H 4.6.

Series IV, 3.5 cc. KOH, giving approximately P_H 5.0.

Series V, 5.0 cc. KOH, giving approximately P_H 6.0.

The sterile acid and alkali were added aseptically. After thoroughly mixing to assure uniform distribution, the solutions were poured into sterile plates, cooled, and inoculated.

Glassware.—All the glassware was scrubbed with cleaning powder, cleaned in a strong cleaning solution recommended by Duggar ('09, page 13), rinsed in tap water, rinsed in distilled water, and drained dry. Proper precautions were taken to protect such cleaned glass from dust. Pipettes or other glassware for use under sterile conditions were dry-sterilized for at least 1 hour at 150–170° C.

Methods and examination of cultures.—Using all precautions against contamination, triplicate cultures were made in 120-cc. Erhlenmeyer flasks, each one containing 35 cc. of solution. With the exception of the nutrient-agar-cellulose series, which were incubated at room temperature, and of the cellulose and peptone series without sugar, which were incubated only at 25° C., all cultures were incubated for 30 days at 15° C., 25° C., and 35° C. The lowest temperature was maintained approximately between 14° C. and 18° C. in a cellar, while the other 2 were maintained accurately by means of thermostats in incubators.

In all cases where possible, examination was made upon the final P_H of the solution and upon the dry weight of the fungus. This weight was obtained by drying the green mat upon previously dried and weighed filter-papers for 2 days at 100–105° C., and by weighing the combined mat and paper. The difference between the weight of the paper and that of the mat and paper is the weight of the mat alone. The triplicate figures obtained were averaged and given as one reading.

All agar-plate cultures were examined every other day for evidence of growth and for clear zones in the agar, indicating utilization of the cellulose. The diametric growth both of the mycelial colony and of the clear zone was recorded in millimeters. Because of the impossibility of separating the mat from the cellulose and filter-paper, the weights of the fungus in solutions
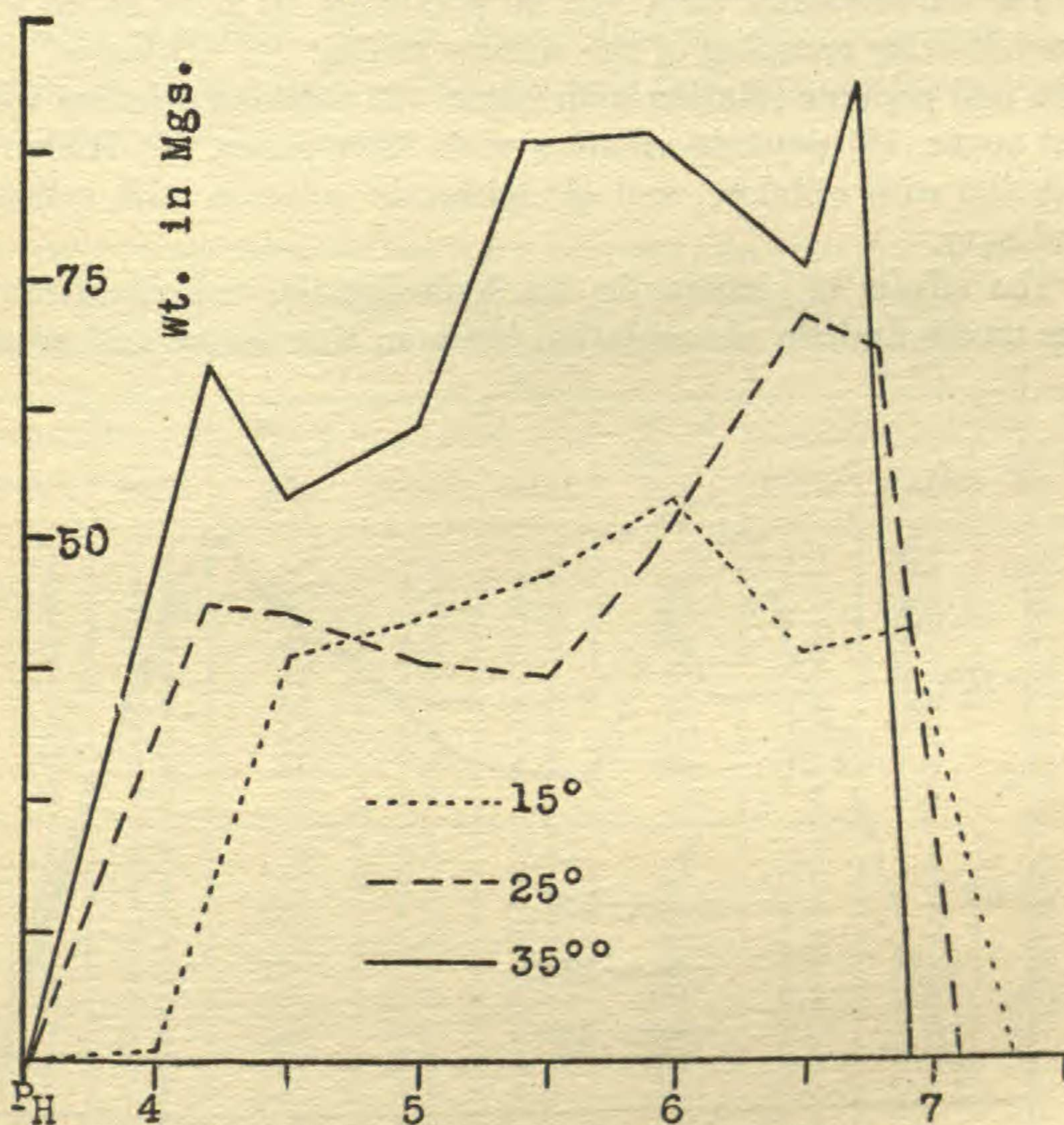
Fig. 1. *Lenzites sepiaria* in Richards' solution.

containing these materials were not determined. In these cases the amount of growth and the hydrolysis of the filter-paper were compared by definite scales. These will be discussed in the experimental data.

As far as possible the work has been reported in the form of curves. The dry weights in mgs. are plotted as the ordinates, and the initial P_H as the abscissae. Unless otherwise stated,

each figure represents a single fungus. In order to keep the size
of the curves within reasonable limits, the ordinates have been
given different unit values, in some cases a unit being 25 mgs., in
some, 50 mgs., and in 2 cases, 100 mgs.

EXPERIMENTAL DATA

The experimental data will be presented in accordance with
the following grouping of the culture media: (1) Richards' solu-
tion and peptone solution with sugar, (2) peptone solution with-
out sugar, (3) peptone solution with filter-paper, (4) Richards'
solution with cellulose, and (5) Richards' solution with cellulose
and agar.

The effects of changes in the hydrogen-ion concentration of
the media and the interrelation between this factor and growth

Fig. 2. *Lenzites sepiaria* in peptone-nutrient solution.
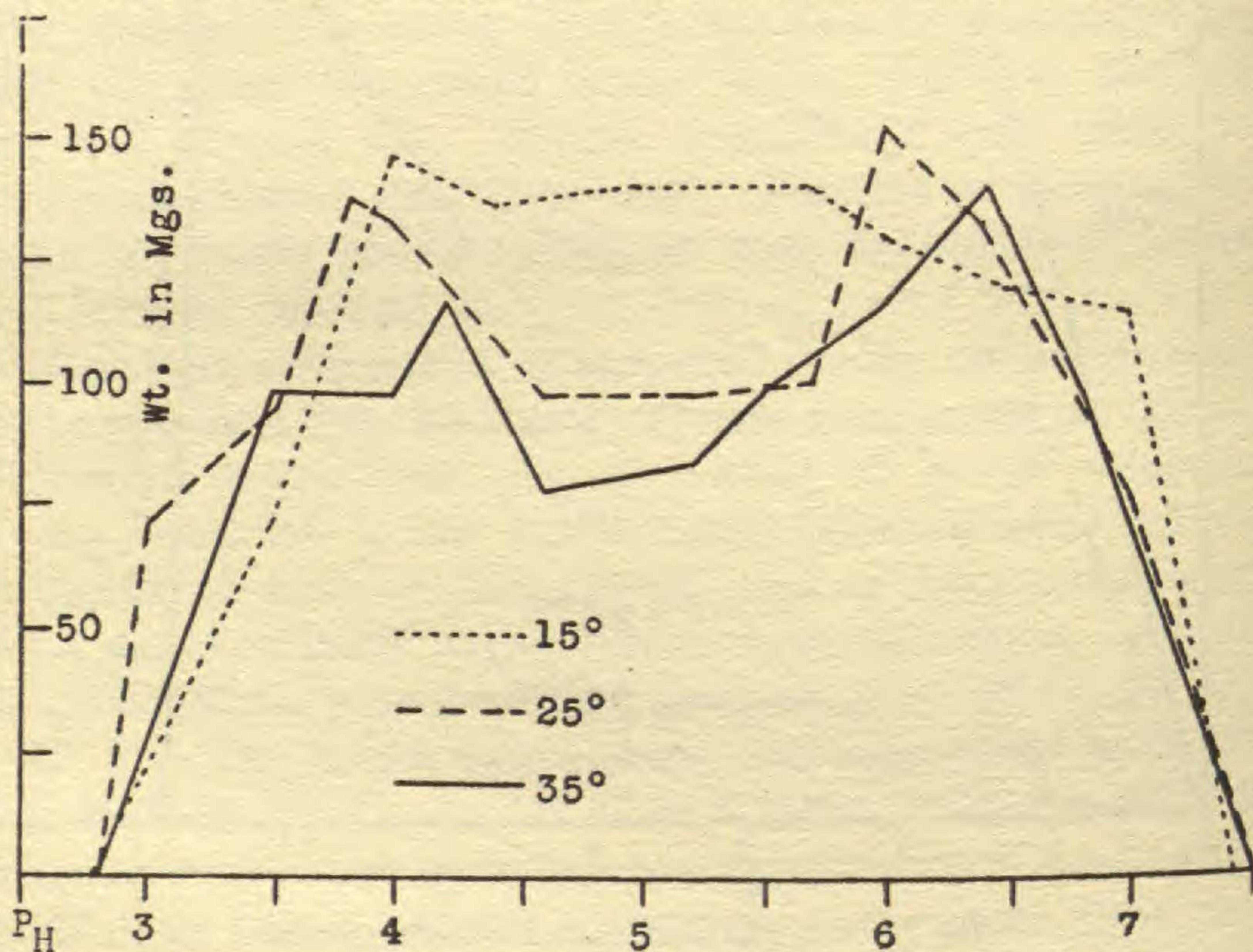
of *Lenzites sepiaria* in the peptone solutions and Richards' solu-
tion are brought out in table IV and figs. 1–2. In the Richards'
solution (fig. 1) the fungus grows slowly. At 15° C. growth is
limited by P_H 3.5 and 7.3 with only a trace[1] at P_H 4.0. It is evi-

[1] Where growth is visible to the eye but too little to be weighed accurately it
is called a trace.

dent that the actual acid limit for appreciable growth lies closer to P_H 4.0 than to 3.5. The best growth is obtained at P_H 6.0 with a mat weighing 54 mgs. At 25° C., although growth is better than at 15° C., it has about the same range of P_H while the optimum lies at 6.5 with 72 mgs. of felt. At 35° C. the fungus grows better than at either of the other temperatures and produces a mat weighing 94 mgs. at P_H 6.7. At this temperature, however, there is no indication of increased tolerance to either alkalinity or acidity. In fact, P_H 6.8 does not support growth as it does at 15° C. and 25° C., while the acid limit remains the same as at the lower temperatures.

TABLE IV

THE GROWTH OF LENZITES SEPIARIA AND THE CHANGES IN THE ACTIVE
ACIDITY IN BOTH THE RICHARDS' AND THE PEPTONE SOLU-
TIONS AT DIFFERENT INITIAL Ph AND TEMPERATURES

	15° C.			25° C.			35° C.		
	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.
	Initial	Final		Initial	Final		Initial	Final	
Richards' sol.	3.5	3.5	0	3.5	3.5	0	3.5	3.5	0
	4.0	3.7	trace	4.2	3.7	44	4.2	3.5	67
	4.5	3.7	39	4.5	3.6	43	4.5	3.8	54
	5.0	3.6	42	5.0	3.6	38	5.0	3.7	61
	5.5	4.4	47	5.5	3.6	37	5.4	3.9	88
	6.0	5.0	54	5.9	4.7	48	5.9	3.9	89
	6.5	5.8	39	6.5	5.0	72	6.5	3.8	76
	6.9	6.4	41	6.8	4.9	68	6.7	7.4	94
	7.3	7.3	0	7.1	7.1	0	6.9	6.9	0
Peptone sol.	2.8	2.8	0	2.8	2.8	0	2.8	2.8	0
	3.5	3.4	71	3.5	3.1	95	3.5	3.2	98
	4.0	3.5	146	3.8	3.1	139	4.0	3.3	98
	4.4	3.3	137	4.6	3.4	99	4.6	3.8	78
	5.0	3.3	141	5.2	3.4	99	5.2	3.6	85
	5.7	3.3	141	5.7	3.5	101	5.5	3.3	100
	6.0	3.4	130	6.0	3.0	153	6.0	3.8	117
	6.5	3.8	120	6.4	3.4	133	6.4	3.6	141
	7.0	4.5	115	7.0	4.0	76	6.8	3.8	99
	7.4	7.4	0	7.6	7.6	0	7.6	7.6	0

In the peptone solution (fig. 2) this fungus grows much better than in the Richards' solution. Growth at 15° C. is as good, if not slightly better, than that at either 25° C. or 35° C. The inhibiting reactions are P_H 2.8 and 7.4. Maximum growth is obtained between P_H 4.0 and 5.7. Although growth is less pronounced at 25° C. than at 15° C., the acid limit is not materially altered.   A

secondary maximum is shown at P_H 3.8 with 138 mgs. This is followed by a marked decrease between P_H 4.0 and 5.5, rising again to the maximum point, P_H 6.0 with 153 mgs. The P_H limits at 35° C. correspond rather closely to those at 15° C. Here, too, there is a secondary maximum at P_H 4.2, and a maximum at P_H 6.4 with 141 mgs. As in the Richards' solution, the optimum hydrogen-ion concentration lies between P_H 5.5 and 7.0.

In all cases growth tends to increase the active acidity of the nutrient solutions (table IV). This is more marked in the peptone
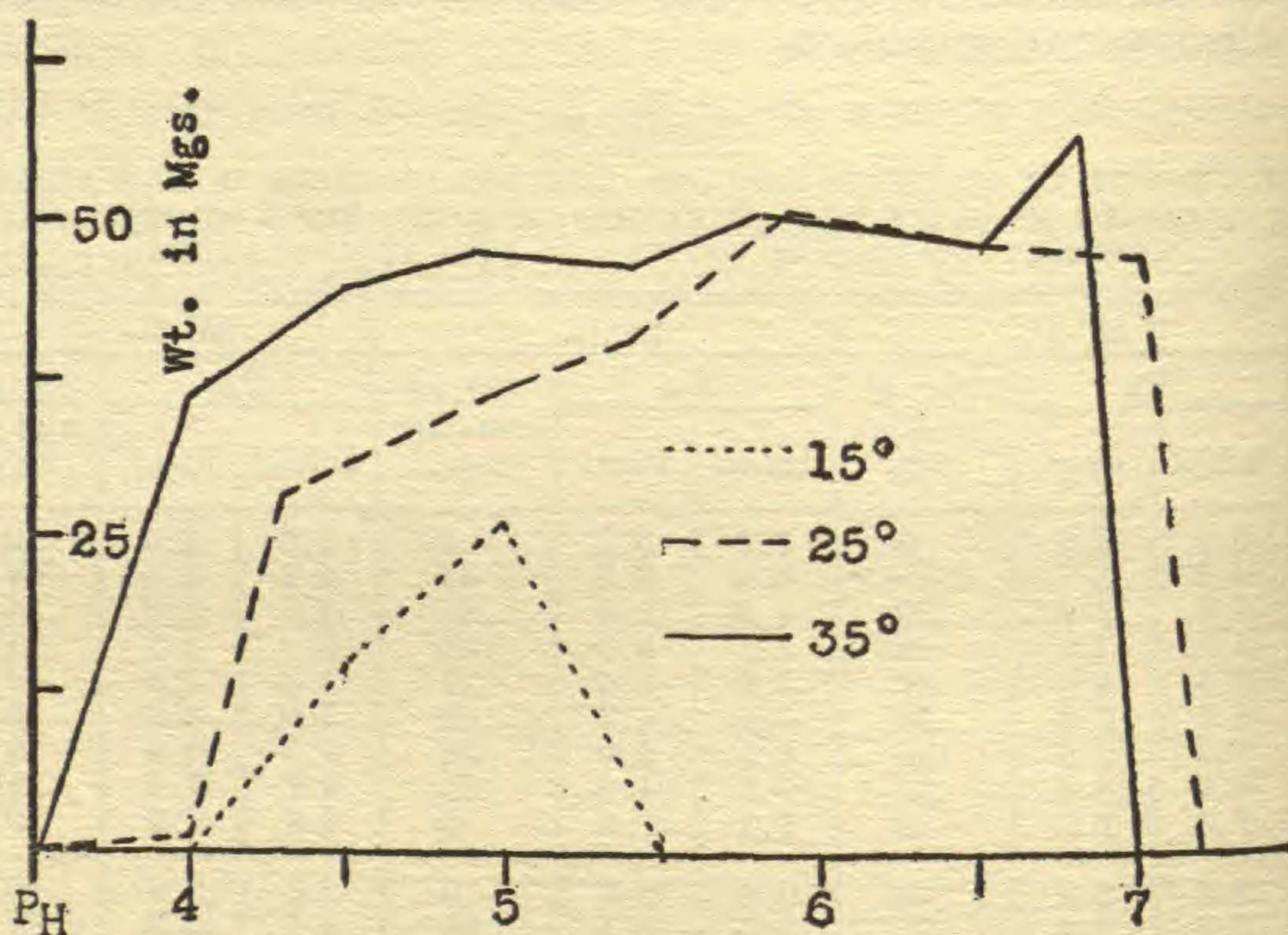
Fig. 3. *Daedalea confragosa* in Richards' solution.

solution where the final P_H range from 3.3 to 4.5 with a mean[1] of 3.5 at 15° C., 3.0 to 4.0 with a mean of 3.3 at 25° C., and 3.2 to 3.8 with a mean of 3.5 at 35° C. In the Richards' solution the final values range from P_H 3.7 to 6.4 with a mean of 4.6 at 15° C., 3.7 to 4.9 with a mean of 4.2 at 25° C., and 3.5 to 4.4 with a mean of 3.9 at 35° C.

The peptone medium supports growth through a slightly wider acid range than does the Richards' solution. However,

[1] The mean is obtained from the final P_H in those solutions supporting mycelia growth.

in neither case does the fungus grow in a slightly alkaline solution. Although 35° C. is the optimum temperature for growth in the Richards' solution, in the peptone it shows no advantage over the other two. The optima P_H ranges do not vary materially in either case except for the secondary maxima found in the peptone solution at 15° C. and 35° C. In both cases the active acidity of the medium is slightly increased. In the majority of instances the final P_H is close to the hydrogen-ion concentration which inhibits the mycelial growth of this fungus.

In the Richards' solution at 15° C. the mycelium of *Daedalea confragosa* (table v, fig. 3) shows a very narrow range of growth, between P_H 4.0 and 5.5. At 25° C. and 35° C. the P_H limit on the acid side is 3.5, but on the alkaline side it is 7.0 at 35° C. and 7.2 for 25° C. The optimum range for the two higher temperatures lies between P_H 5.5 and 7.0, with a slow decrease in growth as the solutions become more acid, and a sharp drop to 0 after passing the neutral point.

TABLE V

THE GROWTH OF DAEDALEA CONFRAGOSA AND THE CHANGES IN THE ACTIVE ACIDITY UPON BOTH THE RICHARDS' AND PEPTONE SOLUTIONS AT DIFFERENT INITIAL Pн AND TEMPERATURES

	15° C.			25° C.			35° C.		
	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.
	Initial	Final		Initial	Final		Initial	Final	
Richards' sol.				3.5	3.5	0	3.5	3.5	0
	4.0	3.9	0	4.0	3.6	trace	4.0	3.7	36
	4.5	3.9	15	4.3	3.5	28	4.5	3.6	45
	5.0	4.7	27	5.0	3.5	37	4.9	3.9	48
	5.5	5.5	0	5.4	3.8	41	5.4	3.8	44
				5.9	4.4	51	5.8	5.1	51
				6.5	5.9	48	6.5	5.7	48
				7.1	6.3	47	6.8	6.3	57
				7.2	7.2	0	7.0	7.0	0
Peptone sol.				2.8	2.8	0	2.8	2.8	0
	3.3	3.3	0	3.5	6.5	332	3.6	6.5	210
	4.0	4.7	166	4.0	6.4	267	4.0	6.6	188
	4.5	5.7	197	4.3	6.7	213	4.2	5.7	128
	4.9	6.0	198	5.0	6.6	212	5.0	6.3	147
	5.5	5.5	150	5.5	6.3	330	5.4	5.5	99
	6.0	5.8	138	6.0	6.3	333	5.9	6.2	110
	6.6	6.2	142	6.4	6.6	197	6.5	5.6	82
	7.0	5.9	100	7.0	6.9	71	7.0	7.1	67
	7.6	7.6	0	7.5	7.5	0	7.5	7.5	0

In the peptone solution (fig. 4) the fungus grows best at 25°
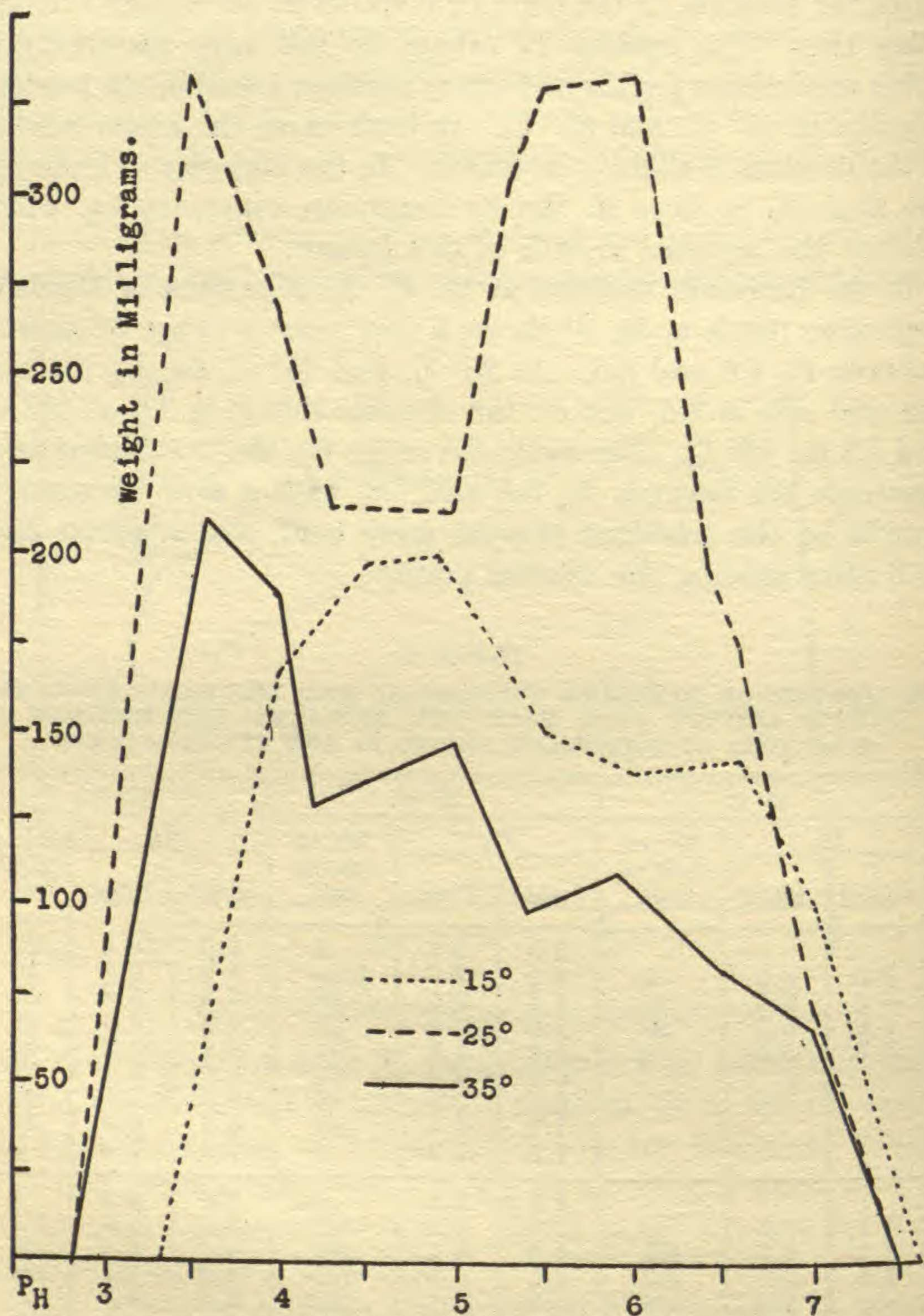C. with maxima of 332 mgs. at P_H 3.5 and of 330 and 333 mgs.

Fig. 4. *Daedalea confragosa* in peptone-nutrient solution.

at P_H 5.5 and 6.0. These two are separated by a sharp drop
at P_H 4.0–5.0. The facts that growth is inhibited at P_H 2.8 at

25° C. and 35° C. and at 3.3 at 15° C., and that the optimum range at 15° C. is P_H 4.5–4.9 and 3.6–4.0 at 35° C. indicate less tolerance to acid at the lowest temperature. The growth curve at 25° C. is the only one to show a secondary maximum toward the neutral point.

In the Richards' solution, in general, the active acidity is increased by growth. The final P_H range from 3.5 to 6.5 at 35° C. with a mean of 5.0, and from 3.6 to 6.3 at 25° C. with a mean
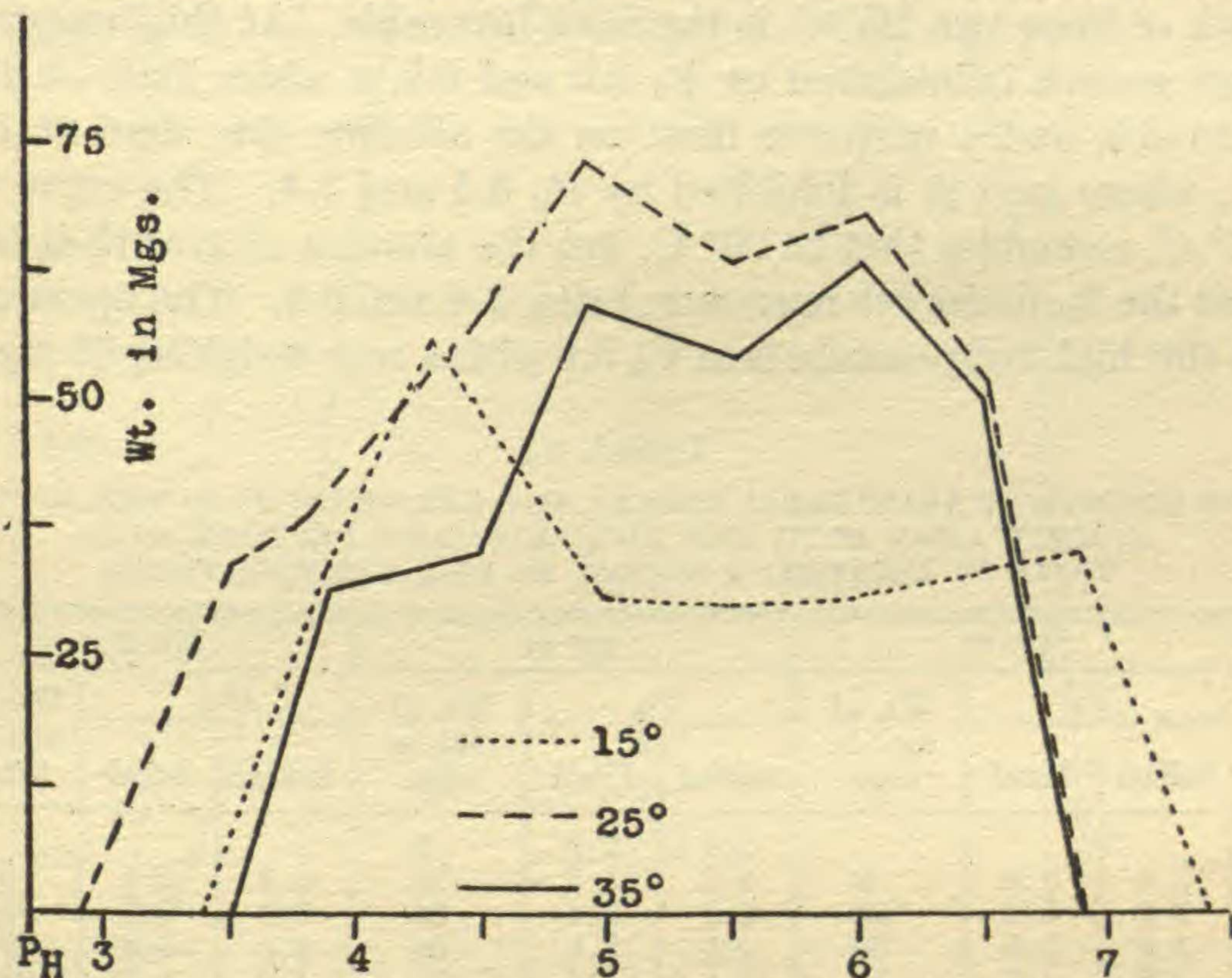
Fig. 5. *Armillaria mellea* in Richards' solution.

of 4.9. On the other hand, in the peptone solution the active acidity is markedly decreased toward neutrality, the final values at 15° C. being P_H 4.7 to 6.9 with a mean of 5.9; at 25° C., 6.2–6.9 with a mean of 6.5; and at 35° C., 5.6 to 7.1, with a mean of 6.3. Only in the initial P_H range, 6.0–7.0 at 15° C., and P_H 7.0 at 25° C. and 6.5 at 35° C. is the acidity of this solution increased.

While this fungus grows a little better at 35° C. than at 25° C. in the Richards' solution, it grows much better at the lower temperature in the peptone solution. In fact, 35° C. is the least favorable of the 3 temperatures in this latter solution, while 15° C. is evidently the poorest in the former. In the peptone, although

the optimum P_H varies with the temperature, it lies in a more acid range than in the Richards' solution. In the first medium the fungus decreases the active acidity close to the neutral point, while in the second, it slightly increases throughout the entire growth-range.

Armillaria mellea in the Richards' solution grows only in cultures in which the reaction is acid, as shown in table vi, fig. 5. Growth at 25° C. and 35° C. is more pronounced than at 15° C. and, of these two, 25° C. is the more favorable. At this temperature growth is inhibited by P_H 2.9 and 6.9, a wider limit on the acid side and a narrower limit on the alkaline side than at 15° C., where growth is inhibited by P_H 3.4 and 7.4. The curve at 35° C. resembles that at 25° C. but the amount of growth is less and the P_H limits are narrower, being 3.4 and 6.9. The optimum in this high temperature is at P_H 6.0 with a mat weighing 63 mgs.,

TABLE VI

THE GROWTH OF ARMILLARIA MELLEA AND THE CHANGES IN THE ACTIVE ACIDITY UPON BOTH THE RICHARDS' AND PEPTONE SOLUTIONS AT DIFFERENT INITIAL P_H AND TEMPERATURES

	15° C.			25° C.			35° C.		
	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.
	Initial	Final		Initial	Final		Initial	Final	
Richards' sol.				2.9	2.9	0			
	3.4	3.4	0	3.5	3.3	34	3.4	3.4	0
	3.9	3.8	34	3.8	3.6	38	3.9	3.7	32
	4.3	3.5	56	4.5	3.9	59	4.5	3.8	35
	5.0	4.5	31	4.9	4.4	74	4.9	4.3	59
	5.5	5.2	30	5.5	5.1	63	5.5	4.9	54
	6.0	5.6	31	6.0	5.7	68	6.0	5.4	63
	6.5	6.2	33	6.5	6.0	52	6.5	6.0	51
	6.9	6.8	35	6.9	6.9	0	6.9	6.8	0
	7.4	7.4	0						
Peptone sol.				2.0	2.0	0			
	2.5	2.5	0	2.5	2.5	19	2.5	2.4	0
	2.8	2.5	70	2.8	2.9	120	3.0	3.3	65
	3.4	5.7	178	3.4	4.0	262	3.6	3.6	139
	4.0	5.7	149	3.8	4.0	301	3.9	4.2	293
	4.5	6.5	105	4.0	3.7	289	4.6	4.2	255
	5.0	6.7	116	4.9	4.8	257	5.0	4.9	271
	5.5	6.8	120	5.3	4.8	252	5.4	5.5	224
	6.0	6.8	115	6.0	4.8	253	6.0	4.6	201
	6.6	6.7	125	6.5	5.0	214	6.4	4.8	281
	7.0	7.4	132	7.0	6.5	112	7.0	5.8	187
				7.2	7.1	79			
	7.5	7.5	0	7.8	7.8	0	7.4	7.4	0

as compared to P_H 4.9 and a mat of 74 mgs. at 25° C. and 4.3 and a mat of 56 mgs. at 15° C.   The optimal P_H for the two higher temperatures is less acid and not as sharply defined as at 15° C.
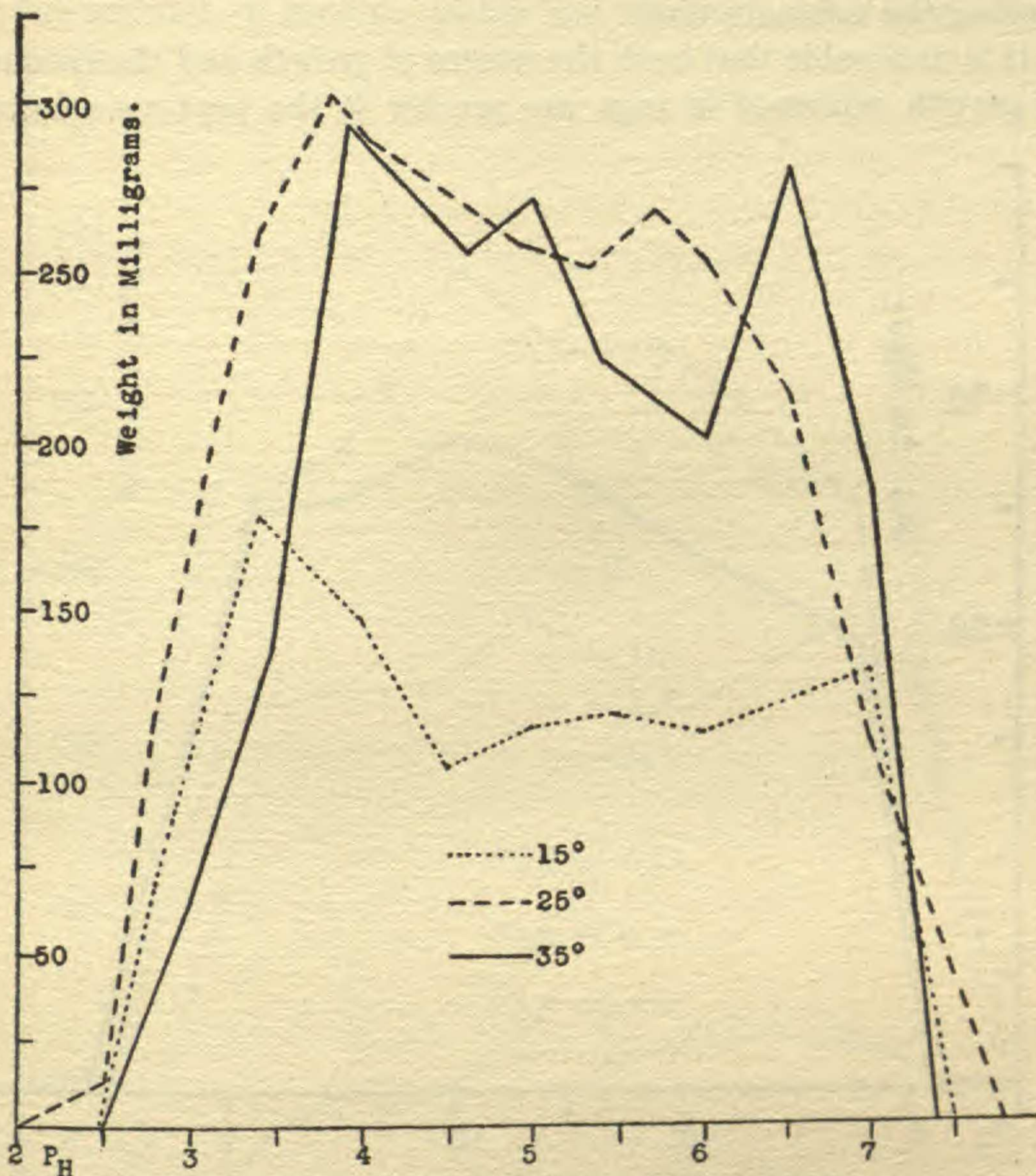
Fig. 6.   *Armillaria mellea* in peptone-nutrient solution.

In the peptone solution (fig. 6) growth at 15° C. is noticeably less than at either 25° C. or 35° C., while at these two temperatures the curves are much alike.   On the other hand, the growth-zones for 15° C. and 35° C. are practically identical, being P_H 2.5–7.5 in the first case and 2.5–7.4 in the second, as compared to 2.0–7.8 at 25° C.   The optimal P_H, 3.4 with a mat weighing 178 mgs. at 15° C., 3.8 with a mat weighing 301 mgs. at 25° C., and 3.9 with a mat weighing 293 mgs. at 35° C., are comparatively

close to the acid limit for growth. In all cases, after passing the optimum, the growth curves gradually and irregularly decrease as the solutions become less acid and fall rapidly to 0 after passing the neutral point.

It is noticeable that both the ranges of growth and the amount of growth expressed in mgs. are greater in the peptone solution
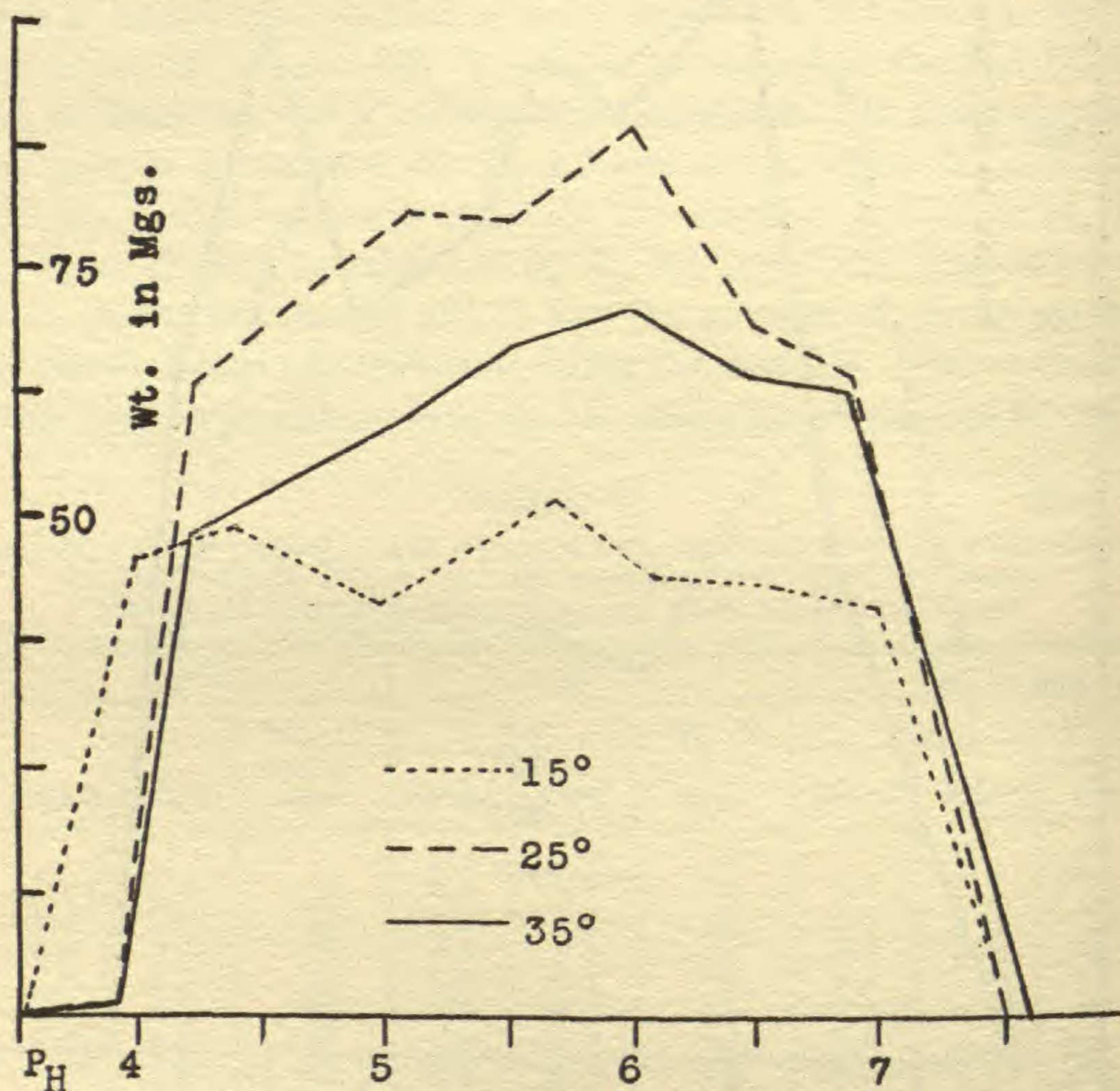
Fig. 7.   *Polyporus adustus* in Richards' solution.

than at the corresponding temperatures in the Richards' solution. In both cases growth at 15° C. is less than that at either 25° C. or 35° C. Of these 2 temperatures, although the differences are not pronounced, 25° C. is better as indicated both by increased growth and by wider P_H limits.

Polyporus adustus (table VII) in the Richards' solution (fig. 7) grows best at 25° C. and least at 15° C. At 25° C. growth is inhibited at P_H 3.3 and 7.5, almost the same as at 35° C. where

growth is inhibited at 3.3 and 7.6.   At 15° C. the inhibiting reactions are P_H 3.5 and 7.5.   At none of the 3 temperatures is there an outstanding maximum, as there is little difference in the amount of growth within the range P_H 4.0–7.0 at 15° C.

Fig. 8.   *Polyporus adustus* in peptone-nutrient solution.

and in the range 4.2–6.0 at 25° C. and 35° C.   The active acidity at 15° C. is not materially changed by growth from the initial P_H; at 25° C. it is slightly decreased through the range P_H 3.9–6.0, and increased at 6.5 and 6.9; while at 35° C. it is decreased slightly at 3.9 and 4.2, and increased in the other solutions from 0.2 to 0.6 of a P_H unit.

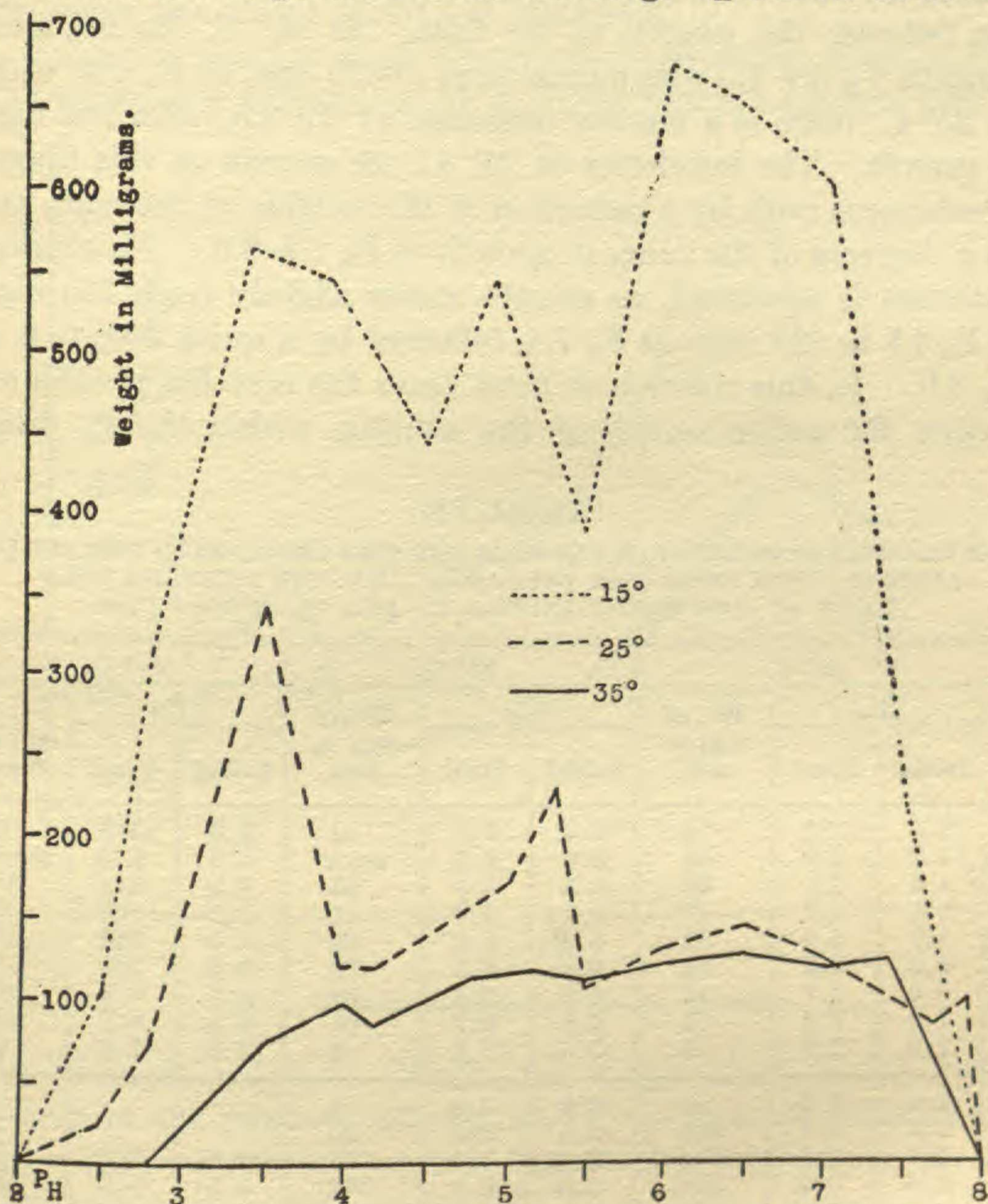The most striking fact in the peptone solution (fig. 8) is the marked superiority of growth at 15° C. over that at either of the other 2 temperatures. Although the range of growth in this case is the same as at 25° C., P_H 2.0–8.0, there is no close comparison between the weights of the felts. At 15° C. the optimum range is P_H 3.4–7.0 with a maximum of 679 mgs. at P_H 6.0, while at 25° C. there is a marked optimum at P_H 3.5, with 342 mgs. of growth. The inferiority of 35° C. for growth of this fungus is evidenced both by a reduction in the weights of the mats and by a decrease of the range of growth to P_H 2.8–8.0. No marked optimum is produced, as growth varies slightly from 115 mgs. at P_H 4.8 to 125 mgs. at P_H 7.4, followed by a quick drop to 0 at P_H 8.0. In this solution in most cases the mycelial growth decreases the active acidity of the solution within the P_H range

TABLE VII

THE GROWTH OF POLYPORUS ADUSTUS AND THE CHANGES IN THE ACTIVE ACIDITY UPON BOTH THE RICHARDS' AND THE PEPTONE SOLUTIONS AT DIFFERENT INITIAL P_H AND TEMPERATURES

	15° C.			25° C.			35° C.		
	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.
	Initial	Final		Initial	Final		Initial	Final	
Richards' sol.	3.5	3.5	0	3.3	3.3	0	3.3	3.3	0
	4.0	3.9	46	3.9	4.3	trace	3.9	4.2	trace
	4.4	4.5	49	4.3	5.3	63	4.2	4.2	48
	5.0	4.4	42	5.1	5.7	81	5.1	4.9	60
	5.7	5.8	52	5.5	5.8	80	5.5	5.4	67
	6.1	6.2	44	6.0	6.3	89	6.0	5.4	71
	6.6	6.7	43	6.5	6.4	69	6.5	6.3	64
	7.0	7.2	71	6.9	6.7	64	6.9	6.7	62
	7.5	7.5	0	7.5	7.5	0	7.6	7.6	0
Peptone sol.	2.0	2.0	0	2.0	2.0	0			
	2.5	2.4	105	2.5	2.5	26			
	2.8	2.4	316	2.8	3.1	75	2.8	2.8	0
	3.4	3.0	564	3.5	3.9	342	3.5	6.7	78
	3.9	3.9	546	4.0	6.5	122	4.0	6.2	100
	4.5	6.8	443	4.2	6.3	121	4.2	6.4	89
	4.9	5.8	544	5.0	6.6	174	4.8	6.0	115
				5.3	7.4	230	5.2	6.2	119
	5.5	6.8	390	5.5	6.6	109	5.5	5.6	111
	6.0	4.5	679	6.0	6.0	131	6.0	6.0	123
	6.4	4.9	658	6.5	6.0	145	6.5	5.3	129
	7.0	5.0	602	7.0	6.0	126	7.0	7.6	121
	7.5	7.4	220	7.7	7.2	89	7.4	6.6	125
				7.9	7.8	102			
	8.0	8.0	0	8.0	8.0	0	8.0	8.0	0

2.5–5.5 and increases it within the range 6.5–7.9. The reaction P_H 6.0 remains close to the initial acidity. Some exceptions to this generality are found, for at 15° C. the acidity of the solutions in the P_H range 4.5–5.5 is decreased, while in the remainder of the series the active acidity is increased.

Polyporus adustus at all temperatures produces more growth and grows at a wider range of P_H in the peptone solution than in
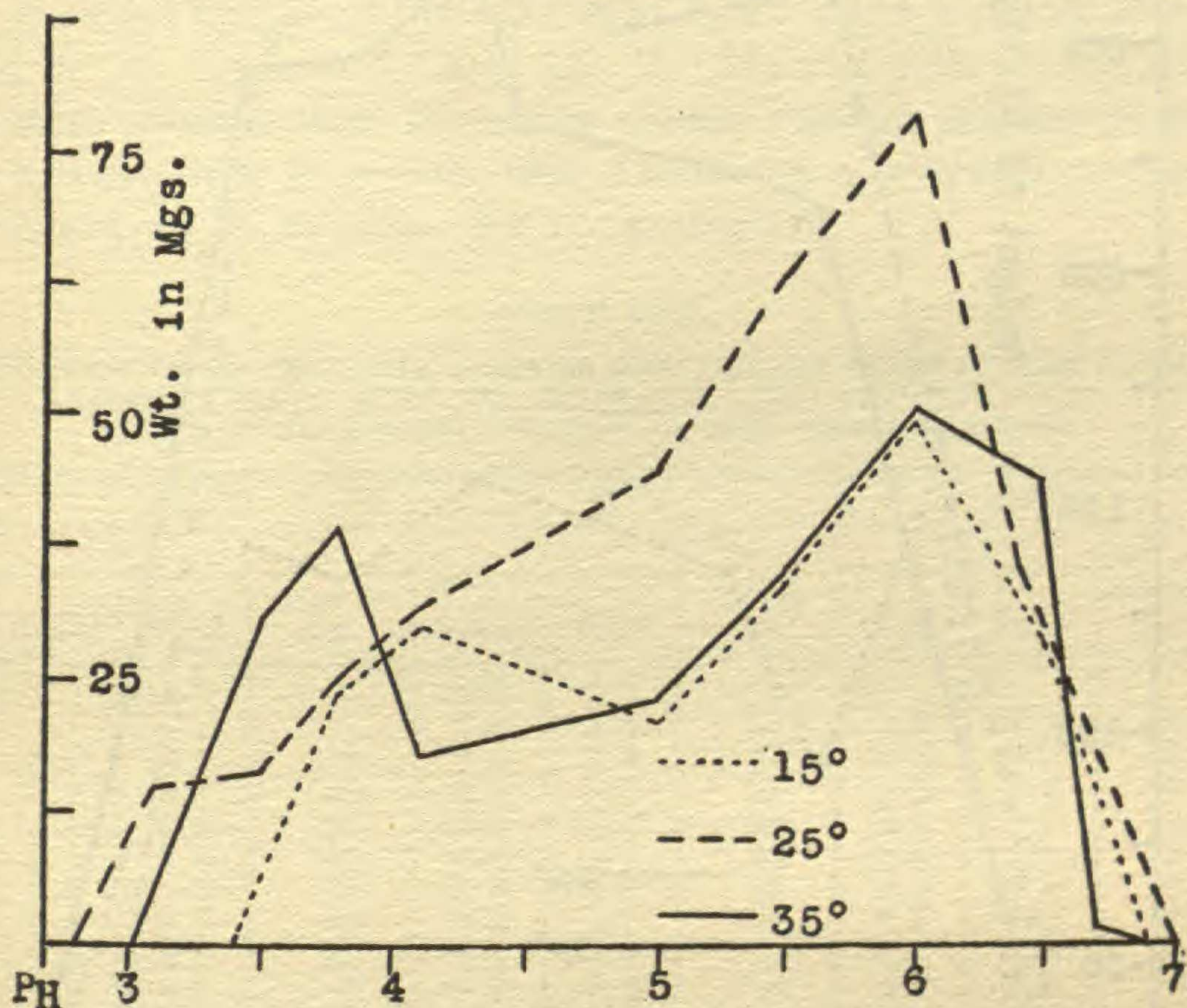
Fig. 9. *Pholiota adiposa* in Richards' solution.

the Richards' solution. While this fungus shows much better growth in the peptone solution at 15° C. than at either of the other 2 temperatures, it grows less at this temperature in the Richards' solution than at either 25° C. or 35° C. Here, however, the variations in growth for the different temperatures are not as marked as they are in the peptone series. As there is no close correlation between the optimal P_H for the two media, since they vary considerably with the temperature and with the solution, it is not possible to designate any definite range of P_H as the optimum for growth of this fungus.

As evidenced by the wider growth limits over those obtained at 15° C. and 35° C., mycelial growth for *Pholiota adiposa* is best at 25° C. as shown in table VIII and fig. 9. Here growth is

Fig. 10. *Pholiota adiposa* in peptone-nutrient solution.

inhibited by P_H 2.8 and 7.0 as compared with 3.4 and 6.9 for both of the other 2 temperatures. Although the growth curves do not vary a great deal through this entire range they indicate that the greatest amount of growth occurred in the cultures incubated at 25° C. The optimum P_H for the 3 temperatures is 6.0.
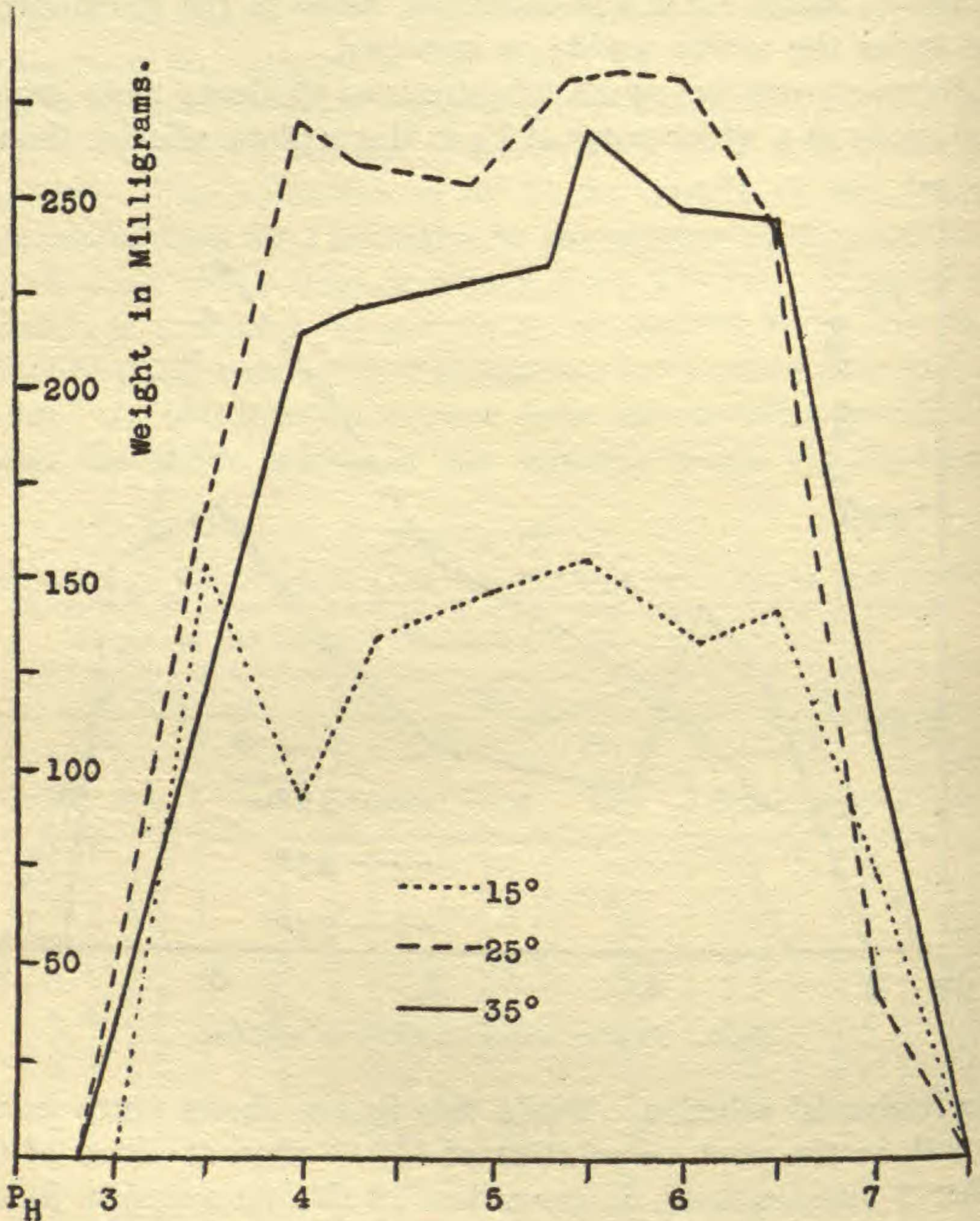
In the peptone solution (fig. 10) the P_H limits at 15° C. are 3.0 and 7.5, while at 25° C. and 35° C. they are 2.8 and 7.5. Here the differences in P_H are slight and point to no marked optimum temperature. On the other hand, growth is much more pronounced at 25° C. and 35° C. than at 15° C., and of these 2, 25° C. is the best. In this solution there is no outstanding P_H indicating an optimum hydrogen-ion concentration, for at 15° C. growth varies very little between P_H 3.5 and 6.5 and at 25° C. and 35° C. between 4.0 and 6.5. In solutions either more acid or more alkaline than the above values, *Pholiota adiposa* shows, by a sharp drop in growth, that it is not on a favorable medium with respect to hydrogen-ion concentration.

TABLE VIII

THE GROWTH OF PHOLIOTA ADIPOSA AND THE CHANGES IN THE ACTIVE ACIDITY UPON BOTH THE RICHARDS' AND PEPTONE SOLUTIONS AT DIFFERENT INITIAL Ph AND TEMPERATURES

	15° C.			25° C.			35° C.		
	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.
	Initial	Final		Initial	Final		Initial	Final	
Richards' sol.				2.8	2.8	0			
				3.1	3.3	15	3.0	3.0	0
	3.4	3.4	0	3.5	3.3	17	3.5	3.2	31
	3.8	3.6	24	3.8	3.6	25	3.8	3.4	39
	4.1	4.0	30	4.1	3.5	32	4.1	3.7	18
	5.0	5.0	21	5.0	4.1	45	5.0	5.0	23
	5.5	5.5	34	5.5	4.8	63	5.5	5.3	35
	6.0	6.0	49	6.0	5.4	78	6.0	5.6	51
	6.5	6.4	28	6.4	6.0	36	6.5	6.0	44
	6.9	6.9	0	7.0	7.0	0	6.9	6.9	0
Peptone sol.	3.0	3.0	0	2.8	2.8	0	2.8	2.8	0
	3.5	3.4	154	3.5	4.2	169	3.5	4.8	122
	4.0	4.9	93	4.0	4.0	268	4.0	6.0	214
	4.4	5.3	135	4.3	4.1	258	4.3	6.0	221
	5.0	6.4	146	4.9	4.1	253	4.9	6.4	228
	5.5	6.9	155	5.4	4.4	275	5.3	6.4	232
	6.1	7.0	133	6.0	4.6	275	6.0	6.3	247
	6.5	7.3	142	6.5	4.6	241	6.5	4.6	244
	7.0	7.2	74	7.0	6.6	44	7.0	6.0	106
	7.5	7.5	0	7.5	7.5	0	7.5	7.5	0

In the Richards' solution the growth of this fungus tends to increase slightly the active acidity (table VIII). However, in no case does this increase amount to more than 1 whole P_H unit while in the majority of cases it is less than one-half of a unit.

In the peptone solution there is a tendency to decrease the active acidity at both 15° C. and 35° C. and to increase it at 25° C. At this last temperature, for solutions within the initial P_H range 4.0–6.5, the final P_H varies from 4.0 to 4.6. Growth at P_H 3.5 tends to decrease the acidity to 4.2. On the other hand, at 35° C. the initial P_H range 4.0–6.0 changes to 6.0–6.3. There is an
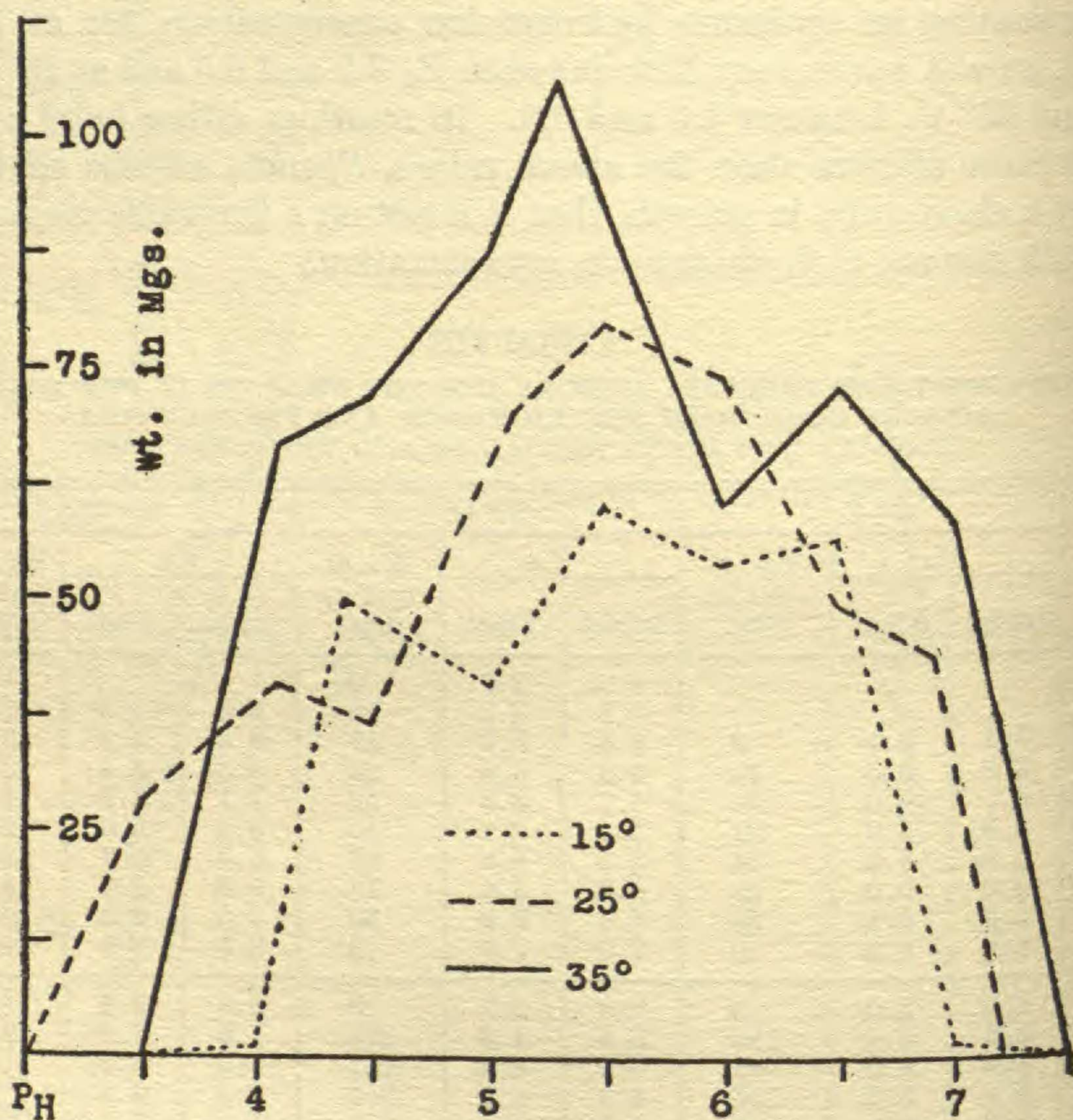
Fig. 11. *Pleurotus ostreatus* in Richards' solution.

increase in the acidity at P_H 6.5 to a final value of 4.6, and a decrease at P_H 3.5 to a final value of P_H 4.8. When one compares the similarity of the growth curves at 25° C. and 35° C. these results are rather unexpected. Throughout the P_H range 4.0–7.0 the decrease in the acidity at 15° C. is less marked than at 35° C.

Upon comparing the growth curves for the 2 solutions, it becomes evident that 25° C. is the optimum temperature of those

employed, and that 35° C. is better on the whole than 15° C. *Pholiota adiposa* also grows better in the peptone solution than in the Richards' solution, producing 4–5 times as much mat in the first solution as is produced in the second. While the fungus grows best at P_H 6.0 in the Richards' medium it does not show any such optimum point in the peptone solution. Furthermore, within the range of these experiments, this fungus does not markedly or invariably decrease or increase the active acidity of the substratum upon which it grows.

The mycelial growth of *Pleurotus ostreatus* (table ix) in the Richards' solution (fig. 11) is limited to a comparatively narrow range of P_H. At 15° C. growth is inhibited at P_H 3.3 and 7.5, but it is evident, however, that the actual limits for growth are nearer to P_H 4.0 and 7.0, as at these values only a trace of growth is obtained. The optimum P_H range at this temperature is 4.4–

TABLE IX

THE GROWTH OF PLEUROTUS OSTREATUS AND THE CHANGES IN THE ACTIVE ACIDITY UPON BOTH THE RICHARDS' AND THE PEPTONE SOLUTIONS AT DIFFERENT INITIAL Ph AND TEMPERATURES

	15° C.			25° C.			35° C.		
	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.
	Initial	Final		Initial	Final		Initial	Final	
Richards' sol.				3.0	3.0	0			
	3.3	3.3	0	3.5	3.3	28	3.5	3.5	0
	4.0	3.7	trace	4.1	3.3	41	4.0	3.7	67
	4.4	4.0	50	4.5	3.3	37	4.5	3.6	72
	5.0	4.0	41	5.1	4.0	70	5.0	4.1	88
	5.5	5.5	61	5.5	4.3	80	5.3	4.1	107
	6.0	6.1	54	6.0	5.3	74	6.0	5.2	60
	6.5	6.8	57	6.5	5.7	49	6.5	5.6	73
	7.0	7.0	trace	6.9	6.2	44	7.0	5.9	58
	7.5	7.5	0	7.2	7.2	0	7.2	7.2	0
Peptone sol.				3.0	3.0	0			
	3.5	3.5	0	3.5	3.5	trace			
	4.1	4.1	46	4.0	6.9	192	4.2	4.2	0
	4.5	4.6	123	4.3	7.0	180			
	5.0	6.4	204	4.9	7.1	136	4.9	5.6	35
	5.5	6.8	194	5.2	6.8	237	5.2	5.8	64
				5.7	6.8	127	5.7	6.3	255
	6.0	7.2	217	5.9	6.7	216	6.0	6.3	207
	6.5	7.3	195	6.4	6.9	142	6.5	7.2	103
	7.0	7.7	155	7.0	7.0	156	7.0	7.9	96
	7.5	8.1	109	7.5	8.4	100	7.5	8.2	90
	8.0	8.0	109	8.0	8.4	86	8.0	8.2	91
	8.5	8.5	0	8.5	8.5	0	8.5	8.5	0

6.5 with maximum growth of 61 mgs. at P_H 5.5 . That 25° C. is more favorable for growth of this fungus than 15° C. is indicated both by the widened range of P_H and by the heavier mats. Here growth is not inhibited until P_H 3.0 is reached on the acid side and until 7.2 is reached on the alkaline side, while best growth is
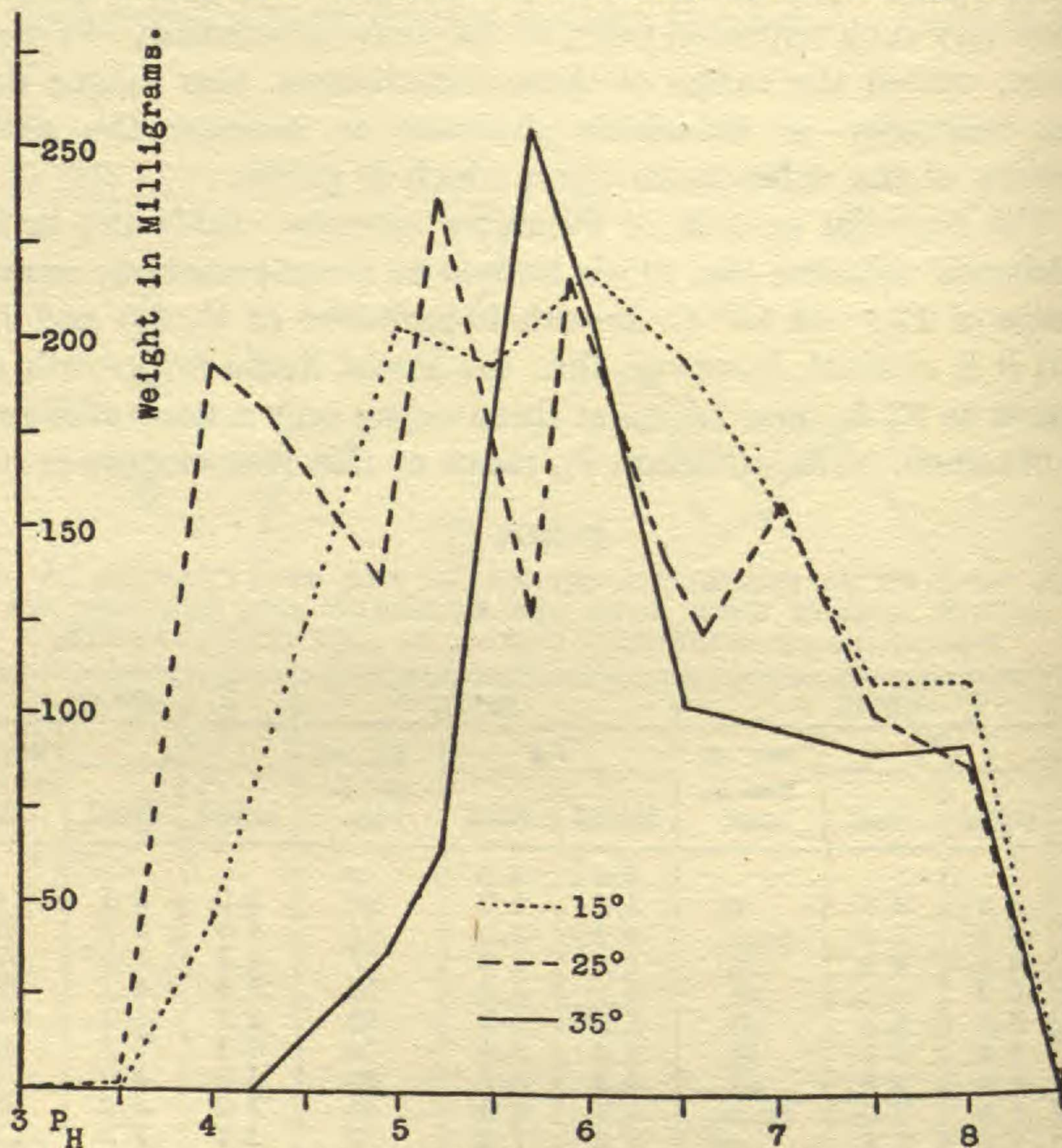

Fig. 12. *Pleurotus ostreatus* in peptone-nutrient solution.

obtained at P_H 5.5 with 80 mgs. Growth of this species is less tolerant to an acid medium at 25° C. than it is at 35° C., but in an alkaline medium, on the other hand, the fungus grows better at the lower of the 2 temperatures. For the 3 temperatures, as indicated by the growth curves, the optimum P_H range is 5.0–6.5.

In the peptone solution (fig. 12), using the amount of growth as an indicator, it is difficult to pick out any optimum temperature,

but from the standpoint of range of P_H, 25° C. is a little better than 15° C. and considerably better than 35° C. Although at this medium temperature the growth curve fluctuates, making it difficult to determine the optimum range, the 2 high points lie at P_H 5.2 and 5.9. At 35° C., however, there is a very sharp optimum of 255 mgs. at P_H 5.7, as compared with 237 mgs. at P_H 5.2, and 216 mgs. at P_H 5.9 for 25° C., and 217 mgs. at P_H
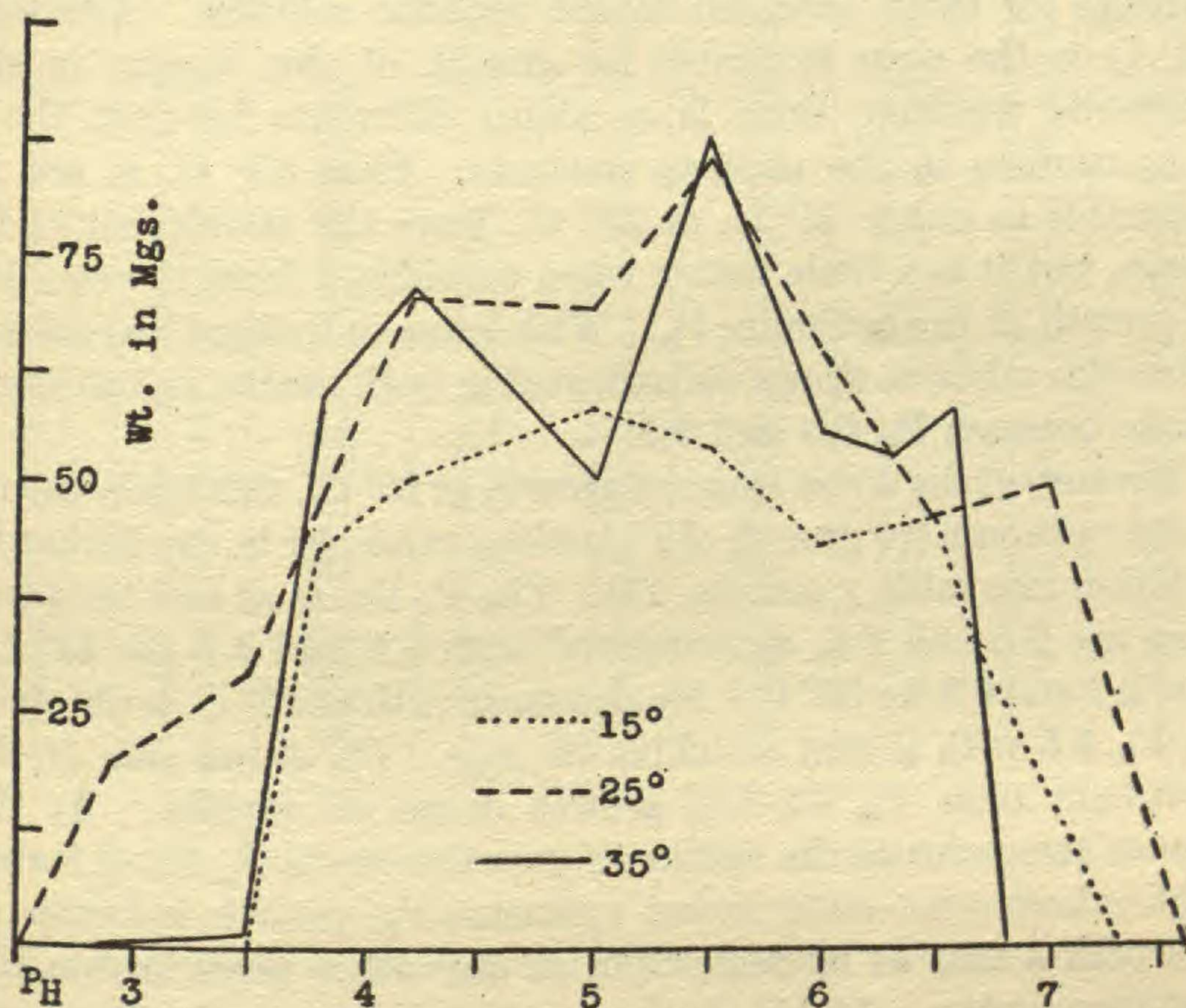

Fig. 13.   *Polystictus versicolor* in Richards' solution.

6.0 for 15° C. Although the fungus may be a little more tolerant to acid at 25° C. than at 15° C., a comparison of the P_H limits and of the optimum range show that there are no fundamental differences in the growth curves of these 2 temperatures.

While the active acidity in the Richards' solution is slightly increased in the majority of cases, in the peptone solution it is invariably decreased close to neutrality or to slight alkalinity. In the former solution, within the P_H range at which *Pleurotus ostreatus* grows, the final P_H at 25° C. varies from 3.3 to 6.2, while in the latter solution the final range at the same temperature

varies from 6.9 to 8.4. This well indicates the different reaction within the 2 types of media.

The marked tolerance to slight alkalinity and the greater amount of growth in the peptone solution show that this medium is much the better of the two. In the Richards' solution there is no indication that this fungus will grow on an alkaline media, while the average weight of the mats is about one-fourth of the average for those obtained in the peptone solution. Although 35° C. is the most favorable for growth of this fungus in the Richards' medium, there is no sharp difference between the 3 temperatures in the peptone medium. Here 35° C. is not as favorable as either 25° C. or 35° C., from the standpoint of P_H range, but it is a little better when considered from the amount of growth at the optimum P_H. With some individual variations, *Pleurotus ostreatus* shows, as indicated in both media, an optimum range between P_H 5.0 and 6.5.

Because of the wider range of growth at 25° C., this temperature is the optimum for growth of *Polystictus versicolor* in the Richards' solution (see table x and fig. 13). The P_H limits at this temperature are 2.5 and 7.6, as compared with 3.5 and 7.3 for 15° C., and 2.9 and 6.8 for 35° C. Maximum growth at 25° C. is obtained at P_H 5.5 with a mat weighing 86 mgs. On either side of the optimum zone, P_H 4.2–6.0, growth drops off rapidly. At the lowest temperature the optimum growth-zone is P_H 3.8–6.5 and, while there is no outstanding optimum P_H, growth is better at 5.0 with a mat of 59 mgs. than at any other point within the optimum range. At 35° C. the fungus produces an optimum at 5.5 with 88 mgs. and an optimum zone between P_H 4.2 and 6.6.

This same fungus in the peptone solution (fig. 14) grows a little better at 15° C. than at 25° C. and much better than at 35° C. At the 2 lower temperatures the P_H limits are practically identical, being 2.5 and 7.4 for 15° C. and 2.5 and 7.5 for 25° C. At 35° C. there is a marked narrowing of the limits on the acid side, the range being P_H 3.0–7.5. At 25° C. and 15° C. marked optima are shown in the growth curves, the first being at P_H 3.5 with 505 mgs. and the second at P_H 4.0 with 507 mgs. The optimum is not so pronounced for 35° C., ranging from P_H 4.0 to 4.9 with 316 and 304 mgs.

TABLE X

THE GROWTH OF POLYSTICTUS VERSICOLOR AND THE CHANGES IN THE ACTIVE ACIDITY UPON BOTH THE RICHARDS' AND THE PEPTONE SOLUTIONS AT DIFFERENT INITIAL P_H AND TEMPERATURES

	15° C.			25° C.			35° C.		
	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.
	Initial	Final		Initial	Final		Initial	Final	
Richards' sol.				2.5	2.5	0			
				2.9	2.9	20	2.9	2.9	0
	3.5	3.5	0	3.5	3.4	30	3.5	3.3	trace
	3.8	3.7	43	3.8	3.5	48	3.8	3.6	60
	4.2	3.6	51	4.2	3.4	71	4.2	3.8	72
	5.0	4.6	59	5.0	3.6	70	5.0	4.2	51
	5.5	5.2	55	5.5	5.0	86	5.5	5.2	88
	6.0	5.9	44	6.0	5.5	65	6.0	5.5	57
	6.5	6.2	47	6.5	5.7	47	6.3	5.5	54
	6.7	6.7	32	7.0	6.2	50	6.6	6.0	59
	7.3	7.3	0	7.6	7.6	0	6.8	6.8	0
Peptone sol.	2.5	2.5	0	2.5	2.5	0			
	3.0	3.0	105	3.0	2.8	50	3.0	3.0	0
	3.5	3.5	402	3.5	4.2	505	3.5	4.4	195
	4.0	5.2	507	4.0	3.8	337	4.0	4.0	316
	4.5	5.6	414	4.2	4.0	320	4.2	4.0	307
	5.0	5.3	441	4.9	4.7	303	4.9	4.8	304
				5.3	5.0	378	5.2	5.0	260
	5.5	5.4	373	5.5	4.9	376	5.5	5.1	273
	6.0	4.5	410	6.0	5.0	395	6.0	5.0	243
	6.5	4.7	374	6.5	4.8	244	6.5	5.0	205
	7.0	5.2	208	7.0	6.7	137	6.8	5.2	119
	7.4	7.4	0	7.5	7.5	0	7.5	7.5	0

This species tends to increase the active acidity of the solutions in which it grows. In every case in the Richards' solution the acidity is slightly increased, usually less than 1 P_H unit. In the peptone solution, with a few exceptions in the more acid range, this tendency persists, the amount of increase, as in the Richard's solution, being less than 1 unit. Comparing the 2 solutions, however, the increase is greater in the peptone solution.

When comparing the growth in the 2 media it becomes evident that the peptone solution is much the better. With peptone as the source of nitrogen, *Polystictus versicolor* produces some 5 to 6 times as much mat as when an inorganic salt is the source of nitrogen. The range of growth in the peptone solution is not materially widened on the alkaline side except at 35° C., but on the acid side it is markedly widened for 15° C. and 35° C. For 25° C. it remains the same in both solutions. It is evident that
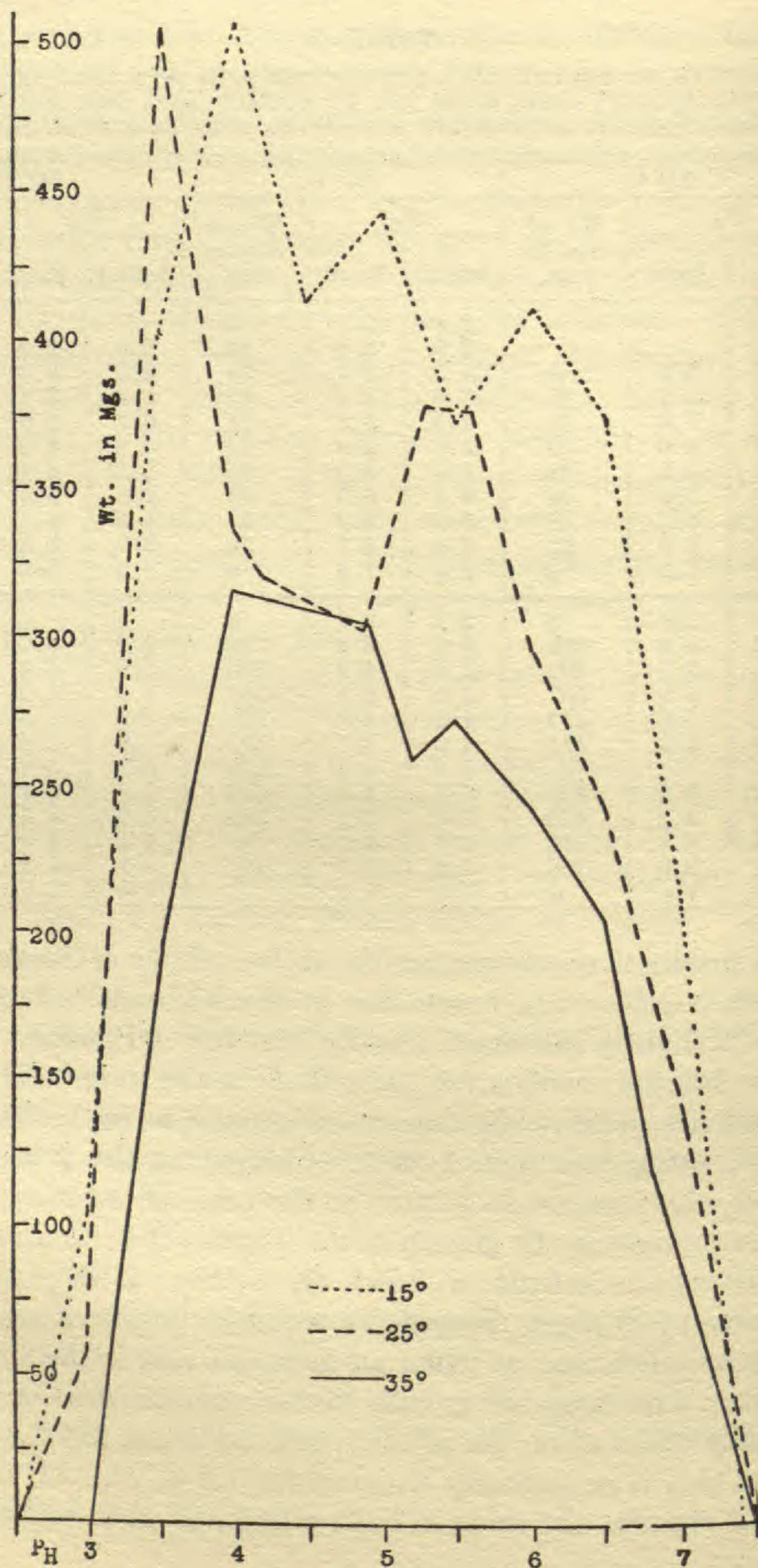
Fig. 14.   *Polystictus versicolor* in peptone-nutrient solution.

the optimum temperature in the one solution is not necessarily the optimum in the other, for in the Richards' solution the fungus shows poorest growth at 15° C., while in the peptone solution it shows best growth at this same temperature.   The poorest results are given at 35° C. in the peptone solution and intermediate in the Richards' solution.   The optimal P_H range in the peptone is
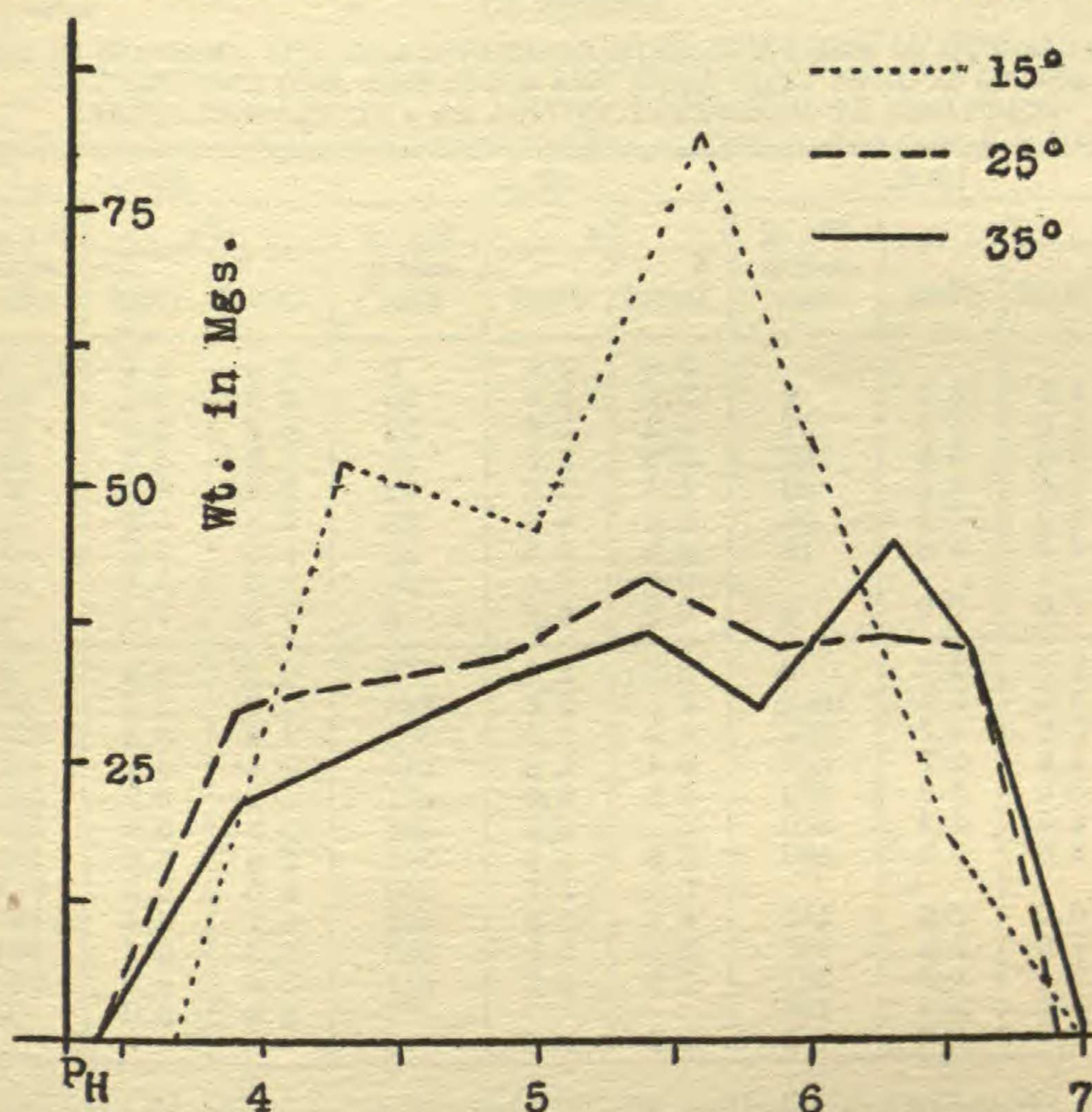

Fig. 15.   *Schizophyllum commune* in Richards' solution.

somewhat more acid than that in the Richards' solution, in the first case being close to the zone P_H 3.5–5.0 and in the second, to the zone 4.0–6.5.   Except at the 2 lower temperatures in the peptone solution, there is no indication of a marked optimum P_H.

Although the P_H range at 15° C. is somewhat narrower than at the other 2 temperatures, *Schizophyllum commune* (table XI) grows best at this temperature in the Richards' solution (fig. 15). Here the P_H limits are 3.7 and 7.0, while at 25° C. and 35° C.

they are 3.4 and 6.9 and 3.4 and 7.0. Only at 15° C. is there any indication of a pronounced optimum P_H, this being 5.6 with 82 mgs. of growth. At the 2 higher temperatures the optimum growth-zone is P_H 4.9–6.5, while at 15° C. it is somewhat narrower, being 4.3–6.0.

TABLE XI

THE GROWTH OF SCHIZOPHYLLUM COMMUNE AND THE CHANGES IN THE ACTIVE ACIDITY UPON BOTH THE RICHARDS' AND THE PEPTONE SOLUTIONS AT DIFFERENT INITIAL P_H AND TEMPERATURES

	15° C.			25° C.			35° C.		
	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.	P_H		Wt. of mat in mgs.
	Initial	Final		Initial	Final		Initial	Final	
Richards' sol.				3.4	3.4	0	3.4	3.4	0
	3.7	3.7	0	3.9	3.6	30	3.9	3.7	22
	4.3	3.7	52	4.2	3.6	32	4.3	3.8	26
	5.0	3.8	47	4.9	3.7	35	4.9	3.8	33
	5.6	4.5	82	5.4	3.8	42	5.4	4.6	37
	6.0	5.4	54	5.9	4.6	36	5.8	5.1	30
	6.5	6.4	18	6.3	5.6	37	6.3	5.5	45
				6.6	6.4	35	6.5	5.8	38
	7.0	7.0	0	6.9	6.9	0	7.0	7.0	0
Peptone sol.	2.8	2.8	0	2.8	2.8	0	2.9	2.9	0
	3.5	3.5	trace	3.5	3.6	248	3.5	3.5	97
	3.9	4.7	687	4.0	5.8	569	4.0	5.8	327
	4.6	5.7	844	4.4	6.0	645	4.4	6.1	483
	5.0	5.9	884	4.9	6.0	671	5.0	5.8	388
	5.5	5.9	880	5.4	6.0	645	5.3	5.9	391
	6.0	5.8	866	5.6	6.0	742	5.6	5.9	289
				6.0	5.8	560	6.0	6.3	512
	6.6	5.9	553	6.4	5.9	542	6.7	6.4	465
	7.0	5.9	431	6.9	5.9	550	7.0	6.4	400
	7.6	6.3	391	7.8	6.2	511	7.5	6.5	305
	8.0	6.5	196				8.0	8.0	0
	8.5	8.5	0	8.5	8.5	0			

In the peptone solution (fig. 16) the fungus again shows optimum growth at 15° C. with a range from P_H 2.8 to 8.5. This is a narrower range on the acid side than that for either 25° C. or 35° C. but a wider range on the alkaline side than for 35° C.; the intermediate temperature having a range from P_H 2.8–8.5 and the higher from 2.9 to 8.0. The optimum P_H zone at 15° C. is 4.6–6.0 with a maximum of 884 mgs. at P_H 5.0, and at 25° C. it is 4.4–5.6 with a maximum of 742 mgs. at P_H 5.6. At 35° C. the growth curve fluctuates widely through a range of 200 mgs. between P_H 4.4 and 7.5, making it difficult to show either an

optimum zone or optimum P_H, but better growth is obtained at P_H 6.0 with 512 mgs. than at either of the other 2 high points, P_H 4.4 and 6.7.
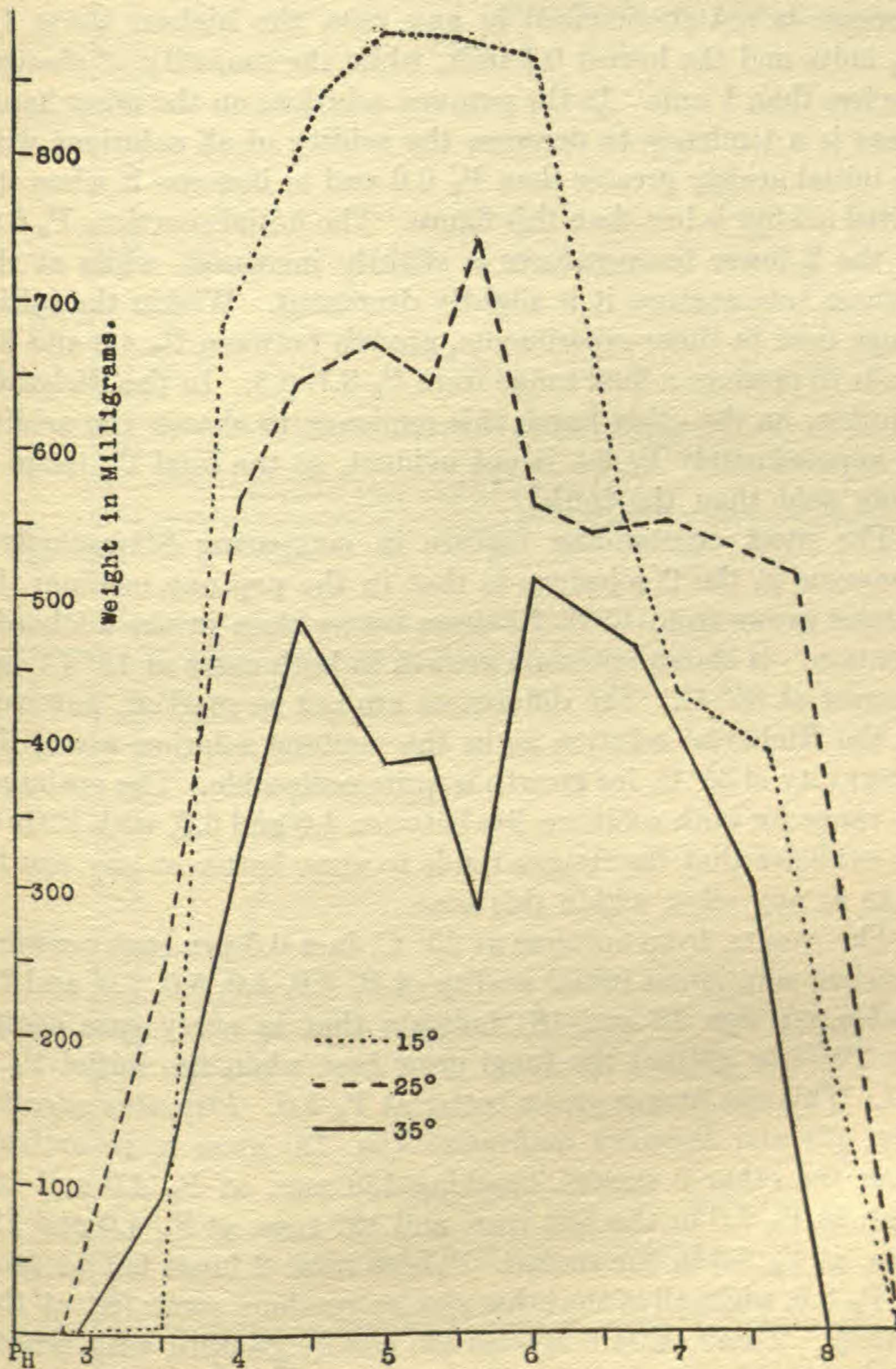

Fig. 16. *Schizophyllum commune* in peptone-nutrient solution.

The mycelial growth of *Schizophyllum commune* tends to increase the active acidity of the Richards' solution throughout the entire P_H range and at all temperatures. The degree of increase is not pronounced in any case, the highest being 1.6 P_H units and the lowest 0.1 unit, while the majority of changes are less than 1 unit. In the peptone solution, on the other hand, there is a tendency to decrease the acidity of all solutions with an initial acidity greater than P_H 6.0 and to increase it when the initial acidity is less than this figure. The initial reaction, P_H 6.0, at the 2 lower temperatures is slightly increased, while at the highest temperature it is slightly decreased. Within the initial range used in these experiments, growth between P_H 4.0 and 8.0 tends to produce a final range from P_H 3.7–6.5. In the Richards' solution, on the other hand, this tendency to change the acidity to approximately P_H 6.0 is not evident, as the final P_H range is more acid than the initial.

The most outstanding feature in comparing *Schizophyllum commune* in the 2 solutions is that in the peptone medium the fungus grows from 15 to 20 times better than in the Richards' solution. It shows optimum growth in both cases at 15° C. and poorest at 35° C. The differences are not so marked, however, in the Richards' solution as in the peptone solution where the inferiority of 35° C. for growth is quite noticeable. The optimum P_H range for both solutions lies between 4.0 and 6.0, with little or no evidence that the fungus tends to grow better at any one P_H than at any other within this zone.

The results, from cultures at 25° C. in a 0.5 per cent peptone-nutrient solution at initial acidity of P_H 3.0, 4.0, 5.0, 6.0, and 7.0 (table XII, figs. 17 and 18), indicate that in every case except for *Pholiota adiposa* the fungi grow best when the initial P_H is 4.1. This one fungus grows better at P_H 5.0. *Pleurotus ostreatus* (fig. 17) and *Daedalea confragosa* (fig. 18) grow more actively than the other 6 species, reaching 139 mgs. at P_H 4.0 and 135 mgs. at P_H 5.0 in the first case, and 127 mgs. at P_H 4.0 and 118 mgs. at P_H 5.0 in the second. These same 2 fungi fail to grow at P_H 3.0, while all of the other species produce some felt at this acidity. These other 6 species are closely grouped with respect to the amount of growth, all being able to utilize peptone as a source of carbon.
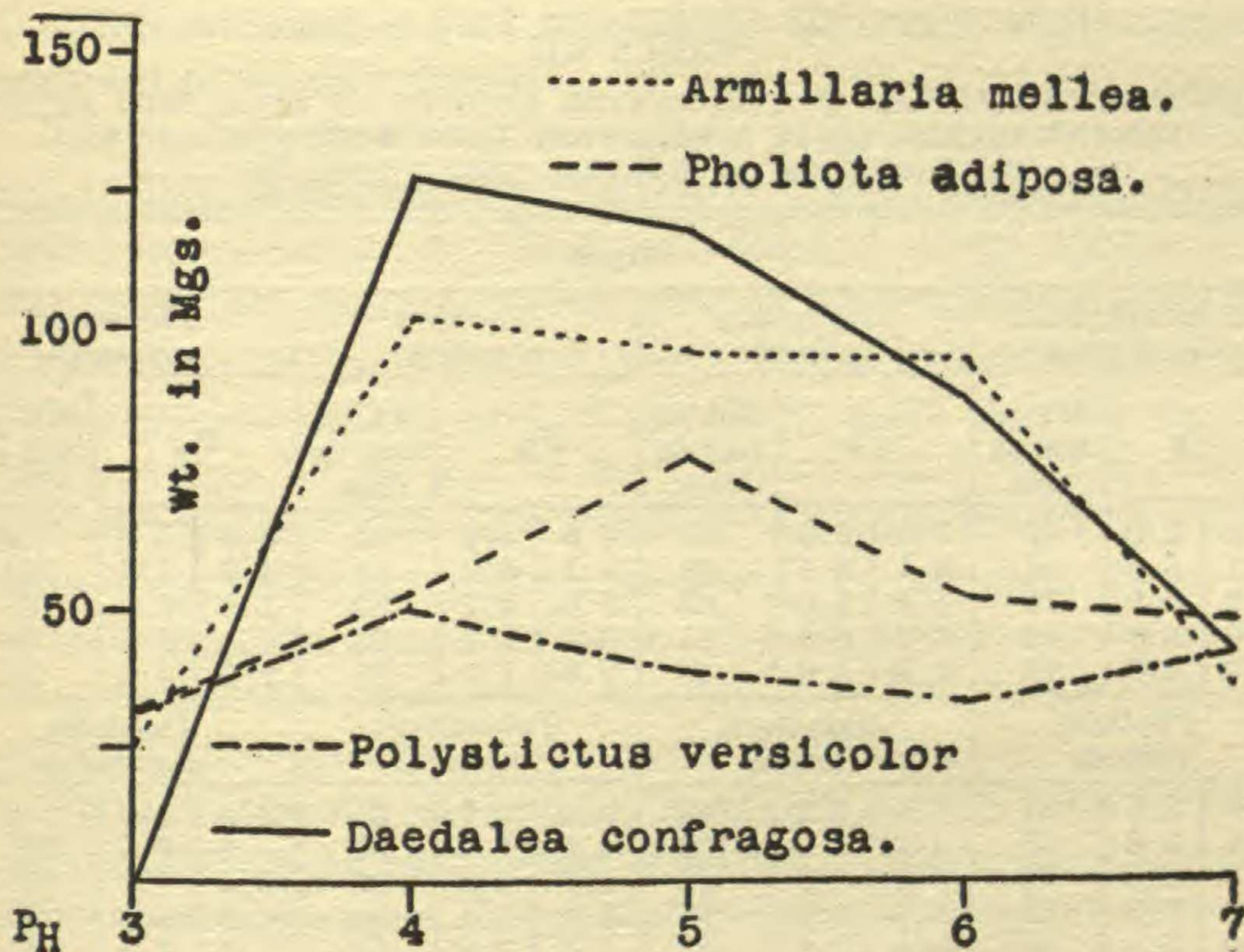
Fig. 17. *Armillaria mellea, Pholiota adiposa, Polystictus versicolor,* and *Daedalea confragosa* in a peptone-nutrient solution without sugar and at 25° C.
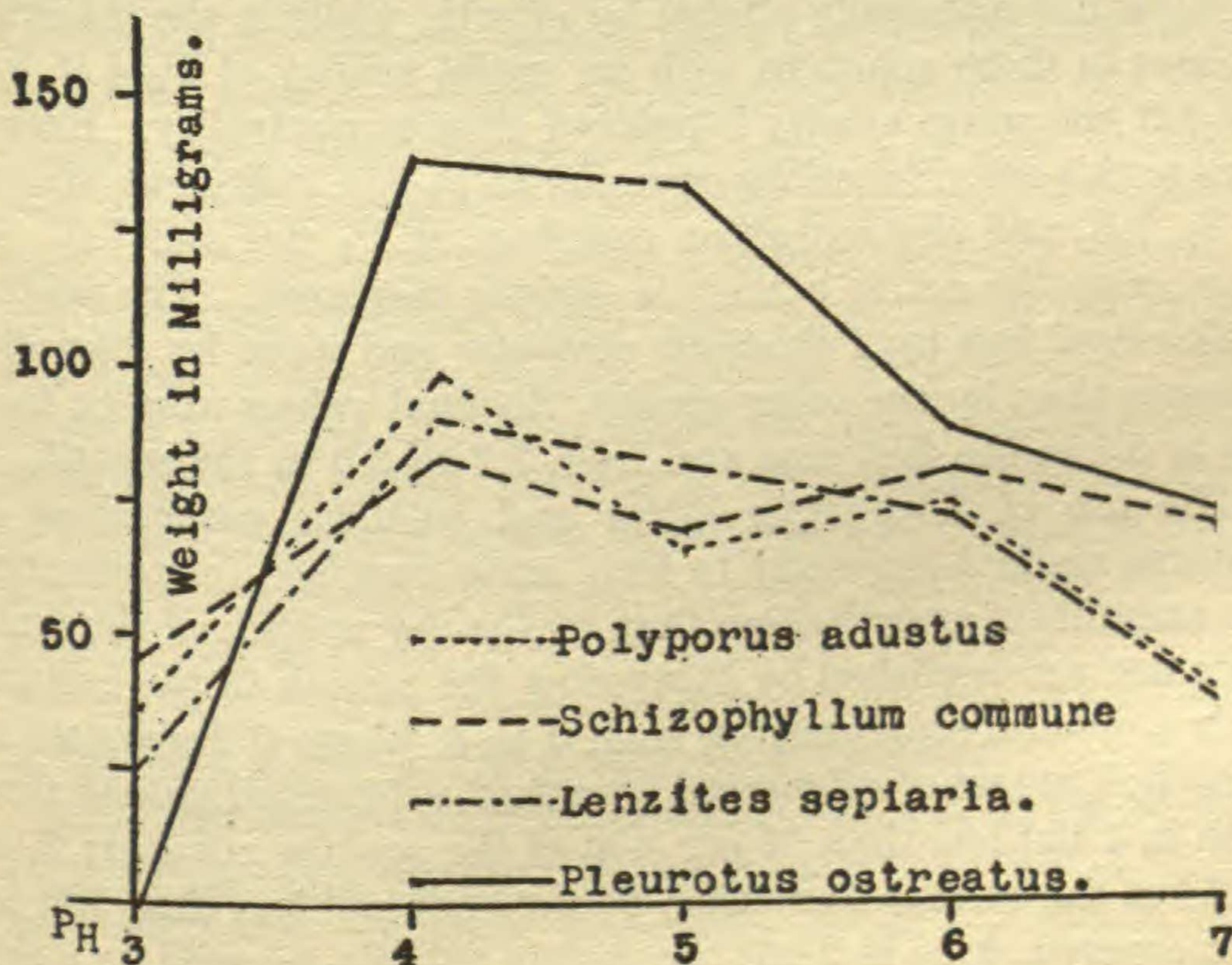

Fig. 18. *Polyporus adustus, Schizophyllum commune, Lenzites sepiaria,* and *Pleurotus ostreatus* in a peptone-nutrient solution without sugar and at 25° C.

TABLE XII

GROWTH AND CHANGES IN THE ACTIVE ACIDITY AT 25° C. AND AT DIF-
FERENT INITIAL Pʜ IN A SOLUTION WITH PEPTONE AS THE
ONLY SOURCE OF CARBON AND NITROGEN

Fungi											
Schizophyllum commune			Polyporus adustus			Lenzites sepiaria			Pleurotus ostreatus		
Pʜ		Wt. of mat in mgs.	Pʜ		Wt. of mat in mgs.	Pʜ		Wt. of mat in mgs.	Pʜ		Wt. of mat in mgs.
3.0	3.0	47	3.0	3.0	37	3.0	3.0	24	3.0	3.0	0
4.1	5.6	84	4.1	6.6	99	4.1	6.6	91	4.0	7.3	139
5.0	5.9	70	5.0	6.0	67	5.0	6.7	82	5.0	7.2	135
6.0	6.0	62	6.0	6.6	75	6.0	7.3	73	6.0	7.9	89
7.0	6.7	70	7.0	6.9	40	7.0	7.2	39	7.0	8.0	78
Pholiota adiposa			Armillaria mellea			Polystictus versicolor			Daedalea confragosa		
3.0	3.5	31	3.0	3.2	25	3.0	3.1	31	3.0	3.0	0
4.0	5.6	53	4.0	6.6	102	4.0	5.4	51	4.0	6.6	127
5.0	6.8	76	5.0	6.7	96	5.0	5.5	39	5.0	6.8	118
6.0	7.0	53	6.0	7.4	95	6.0	5.4	33	6.0	6.9	89
7.0	7.4	49	7.0	7.4	36	7.0	7.5	41	7.0	7.2	41

In every case the active acidity of the solutions with the initial Pʜ 3.0 is not materially altered by growth, while in the majority of cases in those solutions with an initial acidity of Pʜ 4.0, 5.0, and 6.0, the active acidity is reduced close to neutrality. Exceptions to this are: *Polystictus versicolor* at Pʜ 6.0, *Polyporus adustus* at Pʜ 7.0, and *Schizophyllum commune* at Pʜ 6.0 and 7.0. In these cases the active acidity is slightly increased. The acidity is decreased less by *Polystictus versicolor* and more by *Pleurotus ostreatus* than by the other species, the final values ranging from 5.4 to 5.5 in the first case and from 7.2 to 8.0 in the second.

The use of filter-paper strips and cellulose suspensions in cultures made it necessary to form some standard for measuring the amounts of growth other than by the dry weights of the mats. This was accomplished by comparing the growth in the cultures with a definite scale ranging from 0 to 5, where 0 designates no growth; 1, traces of submerged growth; 2, submerged growth more than in 1 but less than 50 per cent of the surface covered; 3, as in 2 but the surface more than 50 per cent and less than 100 per cent covered; 4, as in 2 but the surface entirely covered by a thin film of mycelium; and 5, surface covered by a thicker mat than in

4. This criterion is used throughout the series where celluloses are used in liquid cultures.

The hydrolysis of the filter-paper is also measured according to a definite scale ranging from 0 to 6, where 0 represents no utilization as evidenced by the intact strips; 1, strips appear intact but are rather easily shredded upon being touched; 2, more than 75 per cent of the strips are intact, but 25 per cent are already partially decomposed and shredded; 3, more than 50 per cent and less than 75 per cent of the strips are intact; 4, more than 25 per cent but less than 50 per cent of the strips are intact and less than 25 per cent completely shredded; 5, less than 25 per cent of the strips are intact, but more than 25 per cent and less than 50 per cent are completely decomposed; and 6, the strips are entirely decomposed into a fibrous condition.

Table XIII shows that all the fungi are able to some extent to utilize filter-paper as a source of carbon. Some species, such as *Armillaria mellea* and *Polystictus versicolor*, throughout the entire P_H range from 3.0 to 7.0, show considerable hydrolysis of the paper, more than half of the strips being dissolved and partially utilized. Other forms, such as *Daedalea confragosa*, *Pleurotus ostreatus*, and *Polyporus adustus*, show less utilization of the paper cellulose. *Lenzites sepiaria*, *Schizophyllum commune*, and *Pholiota adiposa* make minimum use of this source of carbon. In only one case, *Pholiota adiposa* at P_H 5.0, is over 50 per cent of the cellulose dissolved, while with the other 2 species, *Lenzites sepiaria* and *Schizophyllum commune*, more than 75 per cent of the strips are left intact or are only slightly shredded after 30 days. *Schizophyllum commune*, *Daedalea confragosa*, and *Pleurotus ostreatus* do not grow at P_H 3.0, while all of the other species are capable of a small amount of growth at this acidity. *Polyporus adustus*, *Daedalea confragosa*, *Pleurotus ostreatus*, and *Armillaria mellea* grow as well at P_H 7.0 as at any other P_H, while the other species are characterized by maximum mycelial growth and utilization of the filter-paper at the intermediate reactions.

Although there is some considerable variation in P_H, all species except *Polystictus versicolor* reduce the active acidity of the more acid solutions. *Daedalea confragosa*, *Armillaria mellea*, *Schizophyllum commune*, and *Polyporus adustus* slightly increase the

TABLE XIII

GROWTH AT 25° C. AND UTILIZATION OF FILTER-PAPER AS THE SOURCE
OF CARBON IN A WEAK PEPTONE SOLUTION WITH
DIFFERENT INITIAL P_H

Initial P_H	Fungi											
	Polystictus versicolor			Polyporus adustus			Schizophyllum commune			Lenzites sepiaria		
	Final P_H	Mycelial growth	Paper utilization	Final P_H	Mycelial growth	Paper utilization	Final P_H	Mycelial growth	Paper utilization	Final P_H	Mycelial growth	Paper utilization
3.0	3.2	3	3	5.0	3	1	3.4	0	0	3.6	2	1
4.0	4.6	4	5	5.9	3	1	5.7	5	2	6.2	3	2
5.0	4.5	5	5	5.8	4	3	6.0	5	2	6.2	3	2
6.0	4.7	4	4	5.9	3	3	6.0	3	1	6.7	3	2
7.0	6.2	2	3	5.6	4	4	6.3	2	1	7.8	2	1
	Daedalea confragosa			Pleurotus ostreatus			Pholiota adiposa			Armillaria mellea		
3.0	2.8	0	0	2.8	0	0	5.7	2	1	3.8	4	4
4.0	6.1	4	3	6.0	5	3	5.5	4	2	4.5	5	5
5.0	5.9	4	3	6.1	5	3	5.0	5	3	5.0	5	5
6.0	5.2	5	4	6.6	5	3	6.6	4	2	4.6	5	5
7.0	6.3	3	3	7.7	5	3	7.8	2	1	4.3	4	5

active acidity of the solutions with an initial P_H 6.0 and 7.0. *Pholiota adiposa, Lenzites sepiaria,* and *Pleurotus ostreatus,* on the other hand, tend to change the initial P_H 6.0 toward neutrality and the initial P_H 7.0 to slight alkalinity. The direction and amount of change in the active acidity vary with the fungus under consideration and with the initial P_H of the solution.

Polyporus adustus, Polystictus versicolor, Schizophyllum commune, Lenzites sepiaria, and *Pleurotus ostreatus* grow slowly in the Richards' solution where cellulose from different species of wood is used as the source of carbon (table XIV). Without an exception the bulk of the growth is beneath the surface of the solution in close contact with the cellulose, forming an inseparable mass. In no case is growth obtained at P_H 2.9, while in the majority of instances maximum growth occurs at P_H 5.0 and 6.0. Of the 5 species, *Lenzites sepiaria* makes the poorest growth throughout and fails to grow at all upon cellulose from poplar wood. *Pleurotus ostreatus* and *Polyporus adustus,* on the other hand, show most active growth. In both of these cases growth is less vigorous on pine-wood cellulose than on the other celluloses

from maple, oak, and poplar woods. *Polyporus adustus* grows best on poplar-wood cellulose, and *Pleurotus ostreatus* on cellulose derived from either maple or poplar woods. *Polystictus versicolor* and *Schizophyllum commune*, while growing less vigorously than either of the other 2 species, do not show decreased growth when pine-wood cellulose is used.

In every case the acidity of the solutions is but slightly changed; where the initial is P_H 4.0, the final is 3.5 to 4.1; where the initial is P_H 5.0, the final is 4.3 to 4.2; and where the initial is P_H 6.0, the final is 5.6 to 6.4. In only one case, *Polystictus versicolor* in maple-wood cellulose, is the final acidity increased where the initial is P_H 6.0, while in just one instance, *Polyporus adustus* in poplar-wood cellulose, is the final acidity decreased where the initial is P_H 5.0. There is no indication that any fungus tends to decrease or increase the active acidity with any degree of regularity.

TABLE XIV

GROWTH AT 25° C. AND AT DIFFERENT INITIAL P_H IN A MODIFIED RICHARDS' SOLUTION WITH CELLULOSE AS THE SOURCE OF CARBON

	Fungi									
	Polyporus adustus		Polystictus versicolor		Schizophyllum commune		Lenzites sepiaria		Pleurotus ostreatus	
Initial P_H	Final P_H	Mycelial growth	Final P_H	Mycelial growth	Final P_H	Mycelial growth	Final P_H	Mycelial growth	Final P_H	Mycelial growth
2.9	2.9	0	2.8	0	2.9	0	2.9	0	2.9	0
4.0	3.9	1	4.1	2	3.9	1	4.1	2	3.8	1
5.0	4.5	2	4.5	3	4.5	2	4.8	2	4.6	2
6.0	5.7	2	6.0	2	6.3	2	6.0	1	6.4	3
2.9	2.9	0	2.8	0	3.0	0	3.0	0	2.9	0
4.0	3.9	1	3.6	2	4.0	1	4.0	1	3.8	2
5.0	5.2	2	4.6	3	4.5	2	4.5	2	4.3	4
6.0	6.3	3	5.6	2	6.1	2	6.2	1	6.2	4
2.9	2.8	0	2.9	0	2.9	0	2.9	0	2.9	0
4.0	3.5	1	4.0	1	3.9	1	4.0	1	3.8	1
5.0	4.9	2	4.6	2	4.6	1	4.8	1	4.8	3
6.0	6.0	3	6.2	0	6.2	2	6.2	2	6.2	3
2.9	—	—	—	—	—	—	—	—	—	—
4.0	4.0	2	4.0	1	4.0	0			4.0	1
5.0	5.1	4	4.5	3	4.8	0			5.0	4
6.0	6.2	3			6.0	0				

When this Richards' solution with the same celluloses as the sources of carbon is solidified with 2 per cent agar and inoculated, the diametric growth of these same 5 species of fungi is slow. It is characterized by a very thin superficial layer of mycelium and by clearing of the agar, denoting utilization of cellulose. The growth in diameter, both of the fungi and of the clear zones, was measured every other day for 18 days, when all growth had stopped, due to the drying of the agar. As the intervening measurements simply show successively increasing growth without any striking departures from the normal, the final figures, as obtained at the end of 18 days, are the only ones presented in table xv.

TABLE XV

DIAMETRIC GROWTH OF THE FUNGI AND OF THE CLEAR ZONES IN A MODI-
FIED RICHARDS' SOLUTION SOLIDIFIED WITH 2 PER CENT AGAR
AND WITH CELLULOSE AS THE SOURCE OF CARBON.
MEASUREMENTS GIVEN IN MILLIMETERS

| Initial P_H | Fungi | | | | | | | | | |
| | Polystictus versicolor | | Pleurotus ostreatus | | Schizo-phyllum commune | | Polyporus adustus | | Lenzites sepiaria | |
	Diameter of mycelial growth	Diameter of clear zone	Diameter of mycelial growth	Diameter of clear zone	Diameter of mycelial growth	Diameter of clear zone	Diameter of mycelial growth	Diameter of clear zone	Diameter of mycelial growth	Diameter of clear zone
2.8	0	0	0	0	0	0	0	0	0	0
4.0	70	48	61	25	68	60	75	*	0	0
4.6	80	41	70	74	80	70	90	60	0	0
5.0	26	0	62	65	62	56	80	65	0	0
6.0	65	32	70	65	55	60	80	40	0	0
2.8	0	0	0	0	0	0	0	0	0	0
4.0	0	0	61	22	0	0	52	30	0	0
4.6	0	0	65	35	46	*	75	30	0	0
5.0	0	0	32	*	47	*	74	60	0	0
6.0	0	0	68	*	42	*	65	55	0	0
2.8	0	0	0	0	0	0	0	0	0	0
4.0	0	0	60	*	48	*	58	30	0	0
4.6	78	35	65	78	70	30	75	70	0	0
5.0	70	35	70	65	40	40	76	60	0	0
6.0	62	38	73	72	55	45	77	75	0	0
2.8	0	0	0	0	0	0	0	0	0	0
4.0	0	0	29	0	36	*	21	0	0	0
4.6	0	0	75	40	69	*	85	60	0	0
5.0	0	0	83	50	41	*	70	35	0	0
6.0	0	0	70	58	36	*	0	0	0	0

* No definite clear zone.

In no case is growth secured in the most acid plates, those with an initial P_H of 2.8. *Lenzites sepiaria* does not grow under any condition and apparently is unable to utilize the cellulose contained in the agar. *Polystictus versicolor* grows and utilizes the cellulose from both poplar and maple woods but not from pine and oak woods, while the other 3 species will grow to some extent upon celluloses derived from the 4 types of wood. There is some doubt as to the utilization of cellulose from pine and oak woods by *Schizophyllum commune*, for in this, and in a few others, the plates remained cloudy in spite of the comparatively active growth. *Pleurotus ostreatus*, one of the most active users of cellulose, does not avail itself of the carbon from the pine-wood cellulose as readily as of the other forms of cellulose, while *Polyporus adustus* does not show this difference. Most active growth and hydrolysis are secured in those plates with an initial P_H of 4.6, 5.0, and 6.0. In some cases, however, as with *Schizophyllum commune* and *Polystictus versicolor* on poplar-wood celluloses, growth of the fungi and utilization of the cellulose are as marked at P_H 4.0 as in the less acid plates.

DISCUSSION

All of the fungi used in these experiments show growth through a considerable range of hydrogen-ion concentration. A brief review of the facts, as previously presented, show that the fungi studied are partial to acid media, and that in the majority of cases they fail to grow in slightly alkaline solutions. It can be said for these fungi in general that they are acid-loving organisms, but such a statement does not imply that none of them will grow upon an alkaline solution. In this respect they exhibit some individual differences.

In the Richards' solution, the less favorable of the 2 major solutions used, there is no indication that any of the 8 species will grow in an alkaline culture. *Polyporus adustus* and *Pleurotus ostreatus* do grow at P_H 7.0. While this tendency to grow in a neutral solution is not as marked for *Polystictus versicolor* as for the first 2 species, it does grow better at P_H 7.0 than do the other 5. Although in some cases growth is obtained at P_H 6.8 and 6.9, *Daedalea confragosa*, *Lenzites sepiaria*, *Schizophyllum*

commune, *Pholiota adiposa*, and *Armillaria mellea* fail to grow at P_H 7.0.

This tendency to grow only in an acid medium is less evident in a more favorable nutrient, the peptone solution. Here in every case growth is secured at P_H 7.0, and only 4 species, *Polystictus versicolor*, *Lenzites sepiaria*, *Pholiota adiposa* and *Daedalea confragosa*, fail to grow in an alkaline solution. For these 4 species growth at P_H 7.0 is much less than at more acid values. Three of the remaining 4 species, *Schizophyllum commune*, *Pleurotus ostreatus*, and *Polyporus adustus*, grow definitely on a slightly alkaline solution, the first 2 growing until P_H 8.5 is reached and the third until P_H 8.0 is reached. *Armillaria mellea* in this case shows an intermediate condition similar to that obtained for *Polystictus versicolor* in the Richards' solution. While *Polyporus adustus* and *Pleurotus ostreatus* are tolerant to a neutral substratum in the Richards' solution and to an alkaline substratum in the peptone solution, this is not true for *Schizophyllum commune*, which is distinctly intolerant to neutrality in the first case and more tolerant to alkalinity than any other species in the second.

Of these fungi which were more tolerant to alkali, *Schizophyllum commune* and *Pleurotus ostreatus* are a little less tolerant to acid in the peptone solution than the other species, while *Polyporus adustus* is markedly more tolerant (table xvi). In the Richards' solution this relationship is not as distinct, for, while *Schizophyllum commune* retains indications of being less tolerant to acid, *Pleurotus ostreatus* and *Polyporus adustus* differ little or not at all from the other 5 fungi. On the other hand, of those species which show no indication of growth in alkaline solution, *Lenzites sepiaria* and *Pholiota adiposa* react as do the majority with reference to acid tolerance, while *Polystictus versicolor* shows a wider range on the acid side in the Richards' solution. *Armillaria mellea* exhibits a wider range on the acid side in the peptone solution. In other respects these 2 species do not differ from the majority.

The data obtained from these experiments have shown that mycelial growth of *Polystictus versicolor*, *Lenzites sepiaria*, *Pholiota adiposa*, *Armillaria mellea*, and *Daedalea confragosa* is

TABLE XVI

THE HYDROGEN-ION CONCENTRATION, EXPRESSED IN P_H,
CAPABLE OF INHIBITING MYCELIAL GROWTH

Fungi	Solution											
	Richards'						Peptone					
	Acid limit in P_H			Alk. limit in P_H			Acid limit in P_H			Alk. limit in P_H		
	° C.			° C.			° C.			° C.		
	15	25	35	15	25	35	15	25	35	15	25	35
S. commune	3.7	3.4	3.4	7.1	6.9	7.0	3.5	2.8	2.9	8.5	8.5	8.5
L. sepiaria	3.5	3.5	3.5	7.3	7.1	6.9	2.8	2.8	3.0	7.4	7.5	7.6
Ph. adiposa	3.4	2.8	3.4	6.9	7.0	6.9	3.0	2.8	2.8	7.5	7.6	7.6
P. versicolor	3.5	2.5	3.0	7.3	7.6	6.8	2.5	2.5	3.0	7.4	7.5	7.5
Pl. ostreatus	3.3	2.9	3.5	7.5	7.2	7.5	3.5	3.0	4.2	8.5	8.5	8.5
D. confragosa	4.0	3.5	3.5	5.5	7.2	7.2	3.3	2.8	2.8	7.6	7.5	7.5
P. adustus	3.5	3.3	3.3	7.5	7.5	7.6	2.0	2.0	2.8	8.0	8.0	8.0
A. mellea	3.4	2.9	3.5	7.4	6.9	6.9	2.5	2.0	2.5	7.5	7.8	7.4

checked, or else is very poor, in alkaline solutions, and is inhibited in the acid range P_H 3.0–3.5 in the Richards' solution and 2.5–3.0 in the peptone solution. *Schizophyllum commune* and *Pleurotus ostreatus* are more tolerant to alkaline media and less tolerant to acid media, and *Polyporus adustus* is more tolerant to both alkalinity and acidity than are the above 5 species of fungi. These conclusions agree with those reached by Rumbold ('08), Spaulding ('11), Zeller ('16), and others who have observed that *Lenzites sepiaria* is extremely sensitive to traces of alkalinity. Zeller, Schmitz, and Duggar ('19) reported that *Polystictus versicolor* grew at P_H 8.6 in a Czapek's solution, changing the initial acidity to P_H 4.8. It is evident that fungi respond to wider or narrower ranges of P_H in response to various complex factors. Such a complex and interdependent set of environmental and physiological conditions control the vitality of these fungi that it is difficult to foresee just why divergent results are obtained at different times.

Without an exception the widest optimum P_H range is obtained in the more favorable medium, the peptone solution. Here, with individual variations, the optimum growth-zone is between P_H 3.5 and 6.5. In the less favorable solution, the Richards' solution, the range is more limited, being P_H 4.0–6.0. This does not imply that the optimum range always falls entirely within these limits or that growth is always equally good throughout, but

they do indicate the zones in the major portions of which the fungi show good growth. The optimum range as indicated in any 1 solution does not always foretell the range which will be obtained in any other solution, for if the one solution is more favorable for growth than the other, the range will tend to be widened, producing a curve with a slightly fluctuating optimum zone covering several P_H units.

Furthermore, the optimum range varies slightly with the temperature. A temperature too high or too low for maximum growth tends to affect the physiological balance of the fungus, resulting, no doubt, in a narrower optimum range or a range shifted a little toward either neutrality or greater acidity. Such a case is well illustrated by *Polyporus adustus* (fig. 8), *Polystictus versicolor* (fig. 14), and *Daedalea confragosa* (fig. 4). It is impossible to foretell just how a certain species will react toward a given set of conditions; therefore it is not practicable or even possible to point out any marked optimum P_H or even a narrow range of P_H in which the optimum will invariably fall.

The directions of the changes in the initial acidity due to growth vary with the solution and with the temperature. In general, growth in the Richards' solution tends to increase the acidity. However, *Polyporus adustus* decreases the acidity of this medium in the more acid range, while minor variations from this general increase are to be noted for *Pholiota adiposa* at 15° C. and 25° C. and for *Pleurotus ostreatus* at 15° C. In no case, however, are these exceptions pronounced.

This tendency toward increased acidity is not characteristic of the fungi in the peptone solution. *Lenzites sepiaria* is the only species to increase the acidity throughout the entire P_H range and at all temperatures. *Polystictus versicolor* in general also causes an increase in the acidity of this solution. On the other hand, in marked contrast to its action in the Richards' solution, *Pleurotus ostreatus* decreases the hydrogen-ion concentration in every case. For the other 5 species the results are not uniform, but on the whole the active acidity is decreased within the initial range P_H 2.5–5.5 and increased within the initial range, 6.5–8.0. P_H 6.0 in the majority of cases remains close to the initial, varying little in one direction or the other with the different fungi.

Considering the 2 major solutions, the Richards' and the peptone solution with sugar, *Lenzites sepiaria* is the most active producer of acid, the final P_H in every case being more acid than that produced for the corresponding solution by the other species (table XVII). However, in the peptone solution without sugar and in the Richards' solution with cellulose as the carbon source, this tendency toward greater final acidity is not evident. Table XII shows that in 4 out of 5 cases in the peptone sugar-free solution, the active acidity is actually decreased by *Lenzites sepiaria.*

TABLE XVII

THE AVERAGES OF THE FINAL P_H PRODUCED IN THOSE
SOLUTIONS IN WHICH THE FUNGI GREW

Fungi	Solutions						
	Richards' .			Peptone with sugar			Peptone without sugar
	°C.			°C.			°C.
	15	25	35	15	25	35	25
Pl. ostreatus	5.1	4.5	4.6	6.7	6.9	6.9	6.6
P. adustus	5.5	5.9	5.3	4.6	5.8	6.3	5.8
Ph. adiposa	5.1	4.3	4.6	6.0	5.5	5.9	6.1
L. sepiaria	4.6	4.2	3.9	3.5	3.3	3.5	6.1
S. commune	4.8	4.5	4.6	5.6	5.7	5.9	5.4
P. versicolor	5.1	4.4	4.6	4.8	4.7	4.7	5.4
D. confragosa	4.3	5.0	4.9	5.9	6.5	6.3	6.1
A. mellea	5.1	4.9	4.7	4.6	4.5	6.2	6.2

While *Polystictus versicolor* does not cause such a sharp acid reaction in the substratum upon which it grows, and while the results vary with the medium, this fungus does tend to increase the acidity of all the solutions. The other 6 species are less consistent than these 2 fungi toward increasing the active acidity of the major solutions. Zeller, Schmitz, and Duggar ('19), using 12 species of fungi, found in general that all except *Merulius pinastri* increased the active acidity of a potato broth-nutrient salt solution and that *Polystictus versicolor* increased the active acidity in 7 and slightly decreased it in 4 cases. It is evident that the direction of the change in the initial acidity depends upon a variety of factors.

These factors beyond a doubt are not wholly dependent upon the individual physiological action of the fungus. Undoubtedly the chemical nature and the initial acidity of the substratum

have much to do in determining whether the acidity will be increased or decreased as a result of mycelial growth. Except for *Lenzites sepiaria* and *Polystictus versicolor*, a substitution of peptone with its organic nitrogen for an inorganic nitrogen as well as a reduction in the amount of sugar tends to reduce the acid production by the fungi. Furthermore, while it is not always possible to predict the direction of the changes in acidity, it has been observed in these experiments that those solutions with a low initial acidity become more acid, and those with a high initial acidity become less acid. Temperature, on the other hand, may result in slight variations which are not possible to regulate or to express in any rule. The tendency of *Pholiota adiposa* to increase the active acidity of the peptone solutions at 15° C. and 35° C. and to decrease it at 25° C. well illustrates this point.

It is not possible to draw conclusions showing that those species tolerant to alkalinity produce a low final acidity or that those species tolerant to a more acid substratum produce a high final acidity. *Pleurotus ostreatus* does show an outstanding low final acidity in the peptone solution but not in the Richards' solution, while *Polyporus adustus* and *Schizophyllum commune* do not have a final acidity different from that for the majority of the fungi intolerant to alkalinity. *Lenzites sepiaria*, previously shown to be the most active acid producer under all conditions in the 2 major solutions, shows no tendency to grow on a solution more acid than P_H 3.0.

No one temperature can be shown to be the optimum for all the fungi under all conditions. The same temperature may not be optimum for growth under different sets of conditions. This is well illustrated by *Lenzites sepiaria*, for in the peptone solution it is impossible to indicate any one temperature as the optimum for this fungus, while in the Richards' solution it is evident that 35° C. is the best of the 3. It is more probable that there are optimum ranges of temperature rather than optimum points, and that these ranges vary with the fungi under consideration. Furthermore, these zones may overlap one another and may be widened or narrowed, depending somewhat upon the environmental and physiological factors governing growth. The

species under consideration, however, fall into 3 groups: (1) those fungi which are partial to lower temperatures, as *Schizophyllum commune* and *Polyporus adustus*, (2) those that are partial to intermediate temperatures, as *Pholiota adiposa, Polystictus versicolor*, and *Daedalea confragosa*, and (3) those that are partial to higher temperatures, as *Lenzites sepiaria, Pleurotus ostreatus*, and *Armillaria mellea*.

These temperature relations in culture can be correlated to some extent with the habitat conditions of the fungus in nature. *Schizophyllum commune*, frequently found in the early spring and late fall in shaded brush heaps, grows close to the damp soil and is surrounded by cool moist air. *Polyporus adustus*, often found in the spring and early summer months, grows most frequently on some shaded stump or log where proximity to the soil gives a moist and cool habitat. *Lenzites sepiaria*, one of the species partial to higher temperatures, grows abundantly in the southern states, making its appearance during the warm weather following the rains. For this species, Falck ('09) has found that 35° C. is the optimum. *Polystictus versicolor* is often found growing on stumps during the late spring and early fall months. Bayliss ('08) stated that 15° C. is the most favorable for this fungus, but the results obtained in this study show that there is little to choose between 15° C. and 25° C. It is evident that growth in the peptone solution is a little better at the lower temperature, but the results obtained from the Richards' solution indicate that it will grow equally well at both temperatures.

The peptone solution is by far the best of the different culture media used in these experiments. In every case the fungi show a marked partiality to the organic source of nitrogen, and, as previously mentioned, express this not only in greatly increased growth but also in widened P_H limits and in widened optimum P_H zones. The Richards' solution, on the other hand, is no better than the solution where peptone is the source of both nitrogen and carbon. With this sugar-free medium no effort was made to determine the limits of growth in respect to hydrogen-ion concentration. Consequently, it is not possible to make a sharp comparison, but it is to be noted that with 2 exceptions, *Daedalea confragosa* and *Pleurotus ostreatus*, the fungi grow in

this substratum from P_H 3.0 to 7.0. This is as wide or nearly as wide a range as secured with the majority of the species in the Richards' solution.

The diverse results obtained for the fungi in the solutions at different temperatures emphasize the fact that the wood-destroying fungi do not react alike to any one set of conditions. For this reason it is not feasible to construct a composite curve such as Meacham ('18) has done, showing a maximum, first, and second critical points, and a critical range for different species of fungi. Such a curve suggests that all fungi give the same results in any given set of environmental factors. It is not believed that this is a true assumption. Furthermore, it has been shown that different environmental conditions give different results for the same fungi. Matsumoto ('21), working with strains of *Rhizoctonia*, concluded that the hydrogen-ion concentration gave diverse results in different nutrient solutions because of the probable relations to the availability of the food materials in the different media. Therefore, since any one species of fungus does not necessarily react to a fixed set of environmental factors as would a second species, and since the same fungus reacts differently under different conditions, it is impossible to construct a composite curve representing growth for several fungi in various types of media.

These species of fungi grow to a small extent in a solution where filter-paper strips and a trace of peptone are the sources of carbon. The amount of peptone present in every case is only sufficient to start, but not to maintain, growth. This ability to utilize cellulose is lessened in the Richards' solution when no other source of carbon is provided than cellulose derived from different kinds of wood. Of all the species, *Lenzites sepiaria* and *Schizophyllum commune* are least able to utilize the cellulose in a synthetic culture. Zeller ('16) worked with *Lenzites sepiaria* and found that, on Richards', Colley's "A" and Reed's solutions, and on carrot extract with filter-paper and pine-wood celluloses as the sources of available carbon, it grew very slowly with slight hydrolysis of the pine-wood cellulose but not of the filter-paper cellulose. This distinction was not evidenced in the present cultures, for this species hydrolyzed to a small extent, in a liquid but not

in a solidified media, celluloses derived from the filter-paper and from pine, white oak, and maple woods. Poplar-wood cellulose was not used in either case.

Pleurotus ostreatus, *Polyporus adustus*, and *Armillaria mellea* are equally active in maximum growth and cellulose utilization. While the last species was grown only in culture with the filter-paper strips, its ability to utilize this form of cellulose intimates that it would utilize the celluloses in the Richards' solution as readily as the first 2 species did. The other species show intermediate use of the cellulose between the *Pleurotus ostreatus* type and the *Lenzites sepiaria* type.

It must be remembered that growth in the cellulose-nutrient solutions can not be compared favorably to growth obtained in any of the synthetic media where sugar and peptone are used. In only a few cases was growth equal to that obtained in the Richards' solution. Under the conditions of these experiments there is no doubt that sugar and peptone alone or in combination are much more effective as sources of carbon than any of the cellulose suspensions.

In view of the sensitiveness of many wood-destroying fungi toward alkalinity, it may well be asked if this principle may not be applied in wood preservation. This, of course, is a practical problem beset with many difficulties, such as the diverse conditions under which fungi grow and under which the wood is to be used. However, the inability of many fungi to grow on an alkaline substratum may be of use in the final solution of this problem. A cheap method for impregnation of freshly cut ties and other lumber with some chemical or combination of chemicals, leading to a definite and lasting alkaline reaction of the tissues, would, it is believed, be a definite step in eliminating the heavy financial losses due to the rapid decay of such timber by some species of *Agaricales* and other fungi.

Conclusions

The growth reactions of *Daedalea confragosa*, *Armillaria mellea*, *Pholiota adiposa*, *Pleurotus ostreatus*, *Polyporus adustus*, *Schizophyllum commune*, *Polystictus versicolor*, and *Lenzites sepiaria* toward different initial active acidity of synthetic,

peptone-nutrient, and cellulose-nutrient media at different temperatures have been studied. The limits of P_H, optimum P_H zone, optimum temperature, and changes in the active acidity of the solution due to growth have been determined for each of these species. In addition the utilization by these fungi of strips of filter-paper and of celluloses from white oak, pine, sugar maple, and poplar woods has been studied.

Under the conditions of these experiments it is possible to draw the following conclusions:

(1) The range of P_H in which these fungi will grow and the amount of mycelial growth depend upon the individual organism, the composition of the nutrient solution, the initial active acidity and the temperature.

(2) The major portion of the growth curves of all of these fungi is on the acid side of neutrality and in the majority of cases wholly on the acid side.

(3) In the Richards' solution the P_H which inhibit growth are: *Lenzites sepiaria*, 3.4 and 7.3; *Daedalea confragosa*, 3.5 and 7.2; *Polystictus versicolor*, 2.5 and 7.6; *Armillaria mellea*, 2.9 and 7.4; *Pholiota adiposa*, 2.8 and 7.0; *Polyporus adustus*, 3.5 and 7.6; *Pleurotus ostreatus*, 3.0 and 7.5; *Schizophyllum commune*, 3.4 and 7.0.

(4) Moreover, in the Richards' solution *Polyporus adustus, Schizophyllum commune,* and *Pleurotus ostreatus* grow when the medium is neutral. *Polystictus versicolor* is less tolerant to a neutral solution, while the other 4 species are inhibited by this hydrogen-ion concentration.

(5) In the peptone solution the P_H which inhibit growth are: *Polyporus adustus*, 2.0 and 8.0; *Daedalea confragosa*, 2.8 and 7.6; *Polystictus versicolor*, 2.5 and 7.5; *Armillaria mellea*, 2.0 and 7.8; *Pholiota adiposa*, 2.8 and 7.8; *Lenzites sepiaria*, 2.8 and 7.5; *Pleurotus ostreatus*, 3.0 and 8.5; and *Schizophyllum commune*, 2.8 and 8.5.

(6) In the peptone solution *Schizophyllum commune, Polyporus adustus,* and *Pleurotus ostreatus* grow upon a slightly alkaline solution, while the other 5 species do not.

(7) In the peptone-nutrient solution the fungi grow throughout a wider range of P_H, have a wider optimum P_H zone, and produce more felt than on the Richards' solution.

(8) With the exception of a slight decrease of the initial acidity by *Polyporus adustus* in the more acid solutions, the mycelial growth of all of these fungi increases the acidity of the Richards' solution.

(9) The active acidity of the peptone-nutrient solution is always increased by *Lenzites sepiaria* and decreased by *Pleurotus ostreatus*. The other 6 species tend, with some minor exceptions, to decrease the acidity in solutions where the initial P_H is more acid than 6.0 and to increase the acidity in solutions where the initial P_H is less acid than 6.0.

(10) All the species grow in a medium with peptone as the only source of both nitrogen and carbon. Here growth is as good or even better than in the Richards' solution where carbon is supplied in the form of cane sugar and nitrogen as NH_4NO_3.

(11) These species are capable of utilizing filter-paper strips as a source of carbon in a 0.5 per cent peptone solution: *Polystictus versicolor*, *Armillaria mellea*, and *Pleurotus ostreatus* utilize the cellulose most actively, while *Lenzites sepiaria* and *Schizophyllum commune* utilize it the least.

(12) *Lenzites sepiaria*, *Polystictus versicolor*, *Pleurotus ostreatus*, *Polyporus adustus*, and *Schizophyllum commune* make some use of white oak-, pine-, and maple-wood celluloses, when these are substituted for sugar in the Richards' solution. *Pleurotus ostreatus* and *Polyporus adustus* grow best, and *Lenzites sepiaria* the least, of the 5 species used. While *Lenzites sepiaria* is unable to use cellulose from poplar wood, the other 5 species do use it.

(13) *Polystictus versicolor*, *Pleurotus ostreatus*, *Polyporus adustus*, and *Schizophyllum commune* use these same celluloses in the Richards' solution solidified with 2 per cent agar, while *Lenzites sepiaria* fails to grow under these conditions.

(14) None of the species grow as well in a solution where cellulose is the source of carbon as where sugar and peptone are the sources.

(15) Of the 3 types of liquid media, the peptone-nutrient solution with sugar is by far the best. These fungi appear to make better use of organic forms of nitrogen than they do of the inorganic forms.

(16) It is the belief of the author that under environmental and

physiological conditions other than those in these experiments, the results as here given would be found to vary to some extent. The P_H limits, optimum P_H zone, and direction of change in the active acidity of the substratum vary with the environmental conditions.

The writer wishes to take this opportunity to express his indebtedness to Dr. B. M. Duggar for suggesting this problem and for many kindly and helpful criticisms and suggestions, and to Dr. George T. Moore for the privileges and facilities of the Missouri Botanical Garden.

Graduate Laboratory, Missouri Botanical Garden.

BIBLIOGRAPHY

Bayliss, Jessie S. ('08). The biology of Polystictus versicolor Fr. Jour. Econ. Biol. 3: 1–24. *pl. 1–2.* 1908.

Clark, W. M. ('20). The determination of hydrogen ions. 317 pp. *f. 1–38.* Baltimore, 1920.

————, and Lubs, H. A. ('17). The colorimetric determination of hydrogen ion concentration and its applications in bacteriology. Jour. Bact. 2: 1–34, 109–136, 191–236. *f. 1–9.* 1917.

Duggar, B. M. ('05). The principles of mushroom growing and mushroom spawn making. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 85: 1–60. *pl. 1–7.* 1905.

————, ('09). Fungous diseases of plants. 508 pp. *f. 1–240.* New York, 1909.

Falck, R. ('09). Die Lenzites-Fäule des Coniferenholzes. Möller's Hausschwammforschungen, Heft. 3: 1–234. *pl. 1–7. f. 24.* 1909.

————, ('12). Die Merulius-Fäule des Bauholzes. *Ibid.* Heft. 6: 1–405. *pl. 1–17. f. 73.* 1912.

Matsumoto, T. ('21). Studies in the physiology of the fungi. XII. Physiological specialization in Rhizoctonia Solani Kühn. Ann. Mo. Bot. Gard. 8: 1–62. *f. 1–6.* 1921.

McBeth, I. G. ('16). Studies in the decomposition of cellulose in soils. Soil Science 1: 437–487. 1916.

Meacham, M. R. ('18). Notes upon the hydrogen-ion concentration necessary to inhibit the growth of four wood-destroying fungi. Science N. S. 48: 499–500. *f. 1.* 1918.

Rhoades, A. S. ('21). Some new or little-known hosts for wood-destroying fungi. III. Phytopath. 11: 319–326. 1921.

Richards, H. M. ('97). Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Jahrb. f. wiss. Bot. 30: 665–688. 1897.

Rumbold, C. ('08). Beiträge zur Kenntnis der Biologie holzzerstörender Pilze. Naturwiss. Zeitschr. f. Forst.- u. Landw. 6: 81–140. *pl. 1. f. 1–26.* 1908.

Schmitz, H. ('19). Studies in the physiology of the fungi. VI. The relation of bacteria to cellulose fermentation induced by fungi, with special reference to the decay of wood. Ann. Mo. Bot. Gard. 6: 93–136. 1919.

Spaulding, P. ('11). The timber rot caused by Lenzites saepiaria. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 214: 1–46. *pl. 1–4, f. 1–3.* 1911.

Tubeuf, Carl von ('03). Beiträge zur Kenntnis des Hausschwammes. Naturwiss. Zeitschr. f. Forst. u. Landw. 1: 249–268. *pl. 1–2, f. 1–4.* 1903.

Webb, R. W. ('19). Studies in the physiology of the fungi. X. Germination of the spores of certain fungi in relation to hydrogen-ion concentration. Ann. Mo. Bot. Gard. 6: 201–222. *f. 1–5.* 1919.

—————, ('21). Studies in the physiology of the fungi. XV. Germination of the spores of certain fungi in relation to hydrogen-ion concentration. *Ibid.* **8:** 283–342. *f. 1-39.* 1921.

Wehmer, C. ('14). Die chemische Wirkung des Hausschwamms auf die Holzsubstanz. Ber. d. deut. bot. Ges. **32:** 601–608. 1914.

Weir, J. R. ('14). Notes on wood-destroying fungi which grow on both coniferous and deciduous trees. I. Phytopath. **4:** 271–276. 1914.

Zeller, S. M. ('16). Studies in the physiology of the fungi. II. Lenzites saepiaria Fries, with special reference to enzyme activity. Ann. Mo. Bot. Gard. **3:** 439–512. *pl. 8-9.* 1916.

—————, Schmitz, H., and Duggar, B. M. ('19). Studies in the physiology of the fungi. VIII. Growth of wood-destroying fungi in liquid media. *Ibid.* **6:** 137–142. 1919.